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(54) Title: TRANSGENIC ORGANISM

(57) Abstract

A transgenic organism is described which has an increased starch yield. The preferred embodiment concerns a plant or plant cell containing a recombinant DNA construct containing, in operational relationship to a plant promoter sequence or sequences enabling the expression of the gene by the plant or plant cell thereby to enhance the rate of production and/or yield of starch by the plant or the plant cell, a DNA sequence encoding an exogenous ADP glucose pyrophosphorylase enzyme (AGP) or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme, characterised in that the said DNA sequence is the gene sequence, including non-critical allelic variations thereof, encoding the barley *Hordeum vulgare* endosperm AGP or an active sub-unit thereof, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units.

TRANSGENIC ORGANISM

The present invention relates to a transgenic organism.

In particular, the present invention relates to a transgenic starch producing organism having an increased ability to synthesize starch and one that is capable of producing starch in high yields. More particularly the present invention relates to a transgenic organism comprising a nucleotide sequence coding for exogenous ADP glucose pyrophosphorylase (AGP).

In a preferred embodiment the present invention relates to a transgenic plant or plant cell capable of expressing exogenous AGP in the starch producing centres in the plant, namely the chloroplasts and the amyloplasts. The invention also relates to a recombinant DNA construct for use in the transformation of a plants or plant cell showing enhanced starch production, and plants and plant cells transformed with the recombinant DNA construct.

ADP glucose pyrophosphorylase (E.C.2.7.7.27) (AGP) is one of the primary enzymes involved in the biosynthesis of starch and glycogen in organisms such as plants, algae, fungi and bacteria, particularly plants.

AGP catalyses the reaction:

$$\alpha\text{-glucose-1-P} + \text{ATP} \longleftrightarrow \text{ADP-glucose} + \text{PP}_i$$

the product ADP-glucose being the major donor of glucose in the biosynthesis of starch in plants. Moreover, that reaction has been shown to be the rate limiting factor in the synthesis of starch in organisms such as plants, the rate of that reaction in turn being critically dependent upon the AGP concentration. Because of that, AGP has been the subject of intensive investigation and for a general review of recent studies on plant AGP, reference should be made to Kleczkowski et al: Z. Naturforsch. 46c, 605-612 (1991).

As reported by Kleczkowski et al (*ibid*) and elsewhere, AGP is widely distributed throughout the plant kingdom and is found in some starch producing bacteria, such as E. coli. Plant AGP exists as a tetramer (210 to 240 kDa) composed of two small sub-units (50 to 55 kDa) and two large sub-units (51 to 60 kDa) in contrast to bacterial AGP which appears to consist of four units of equal size. AGP has also been shown to be produced in cyanobacteria and in algae, where its tetrameric structure is similar to that in plants, i.e. two large and two small sub-units, rather than the homotetrameric structure found in ordinary bacteria.

Because of the commercial importance of starch, primarily as a foodstuff bu' also as an important industrial chemical, AGP itself and recombinant DNA constructs containing DNA sequences encoding AGP for the transfection of plants and plant cells as a means of increasing plant AGP concentration and hence increased biosynthesis of starch in plants and increased starch yields, have formed the subject matter of several recently published patent applications.

For example, in EP-A-0368506 a method of extracting AGP from wheat leaf and wheat endosperm is disclosed. Also disclosed are the cDNA sequences encoding wheat leaf and wheat endosperm AGP, and various plasmids containing those sequences for subsequent insertion into plants to provide plants having an increased ability to synthesise starch, although that latter step is not described in detail, nor are any examples given of transgenic plants containing those constructs.

WO 91/19806 discloses transformed plant cells and plants having elevated levels of starch and starch biosynthesis achieved by incorporating into the plant genome a DNA construct comprising in sequence a plant promoter, a DNA sequence encoding a fusion polypeptide consisting of a plastid transit peptide and a bacterial (E. coli) AGP, and a 3'-non-translated region which functions in the plant cell to cause transcriptional termination and the addition of a polyadenylated tail to the 3'- end of the corresponding DNA sequence. The DNA sequence encoding E. coli AGP is given, as well as the deduced amino acid sequence. Transgenic potato and tomato plants transformed with the E. coli AGP gene are shown to produce increased starch

yields. It is suggested that other bacterial sources besides E. coli, and also algae, may be used as a source for the AGP gene to be used in the transformation of the plants and plant cells to provide increased starch yields. However, there is no mention of the isolation of nucleotide sequences coding for AGP enzymes from those other sources or their expression in such transgenic systems.

A similar disclosure is contained in WO 92/11382 which likewise discloses the transformation of plants, especially potato plants, with bacterial (E. coli) DNA encoding bacterial AGP, with the objective of increasing starch biosynthesis and starch yield in such plants.

A slightly different objective is set out in EP-A-0455316. There the objective is to increase sugar and protein concentrations in plant-based foodstuffs at the expense of starch formation. That is achieved by incorporating into the plant genome a DNA sequence encoding AGP, but in an inverted orientation in the transformation vector. Transcription of the reversed sequence results in an anti-sense mRNA which inhibits the production of AGP in the plant cell leading to reduced AGP activity and reduced starch production.

All plant AGPs investigated so far have been reported to be strongly activated by 3-phosphoglycerate (PGA) and inhibited by inorganic phosphate (P_i). Also, the PGA/P_i ratio in the chloroplasts and amyloplasts where biosynthetic starch production is concentrated is believed to play a key regulatory role in starch synthesis. It is known, for example, that chloroplast PGA/P_i ratios are at the highest activity during the daylight hours, i.e. during photosynthesis, which period coincides with the peak period of starch production in the chloroplasts. The regulation of the AGP formation in non-photosynthetic tissues is less well understood, but the activatory and inhibitory roles of PGA and P_i, respectively, i.e. the PGA/P_i ratio, is believed still to play an important part.

The present invention addresses the problem of how to increase AGPase levels and/or starch levels in starch producing organisms.

According to a first aspect of the present invention there is provided a transgenic starch producing organism comprising a nucleotide sequence coding for an exogenous ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme, wherein the nucleotide sequence is capable of being expressed in the organism; characterised in that the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of inorganic phosphate; and further characterised in that the activity of the enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

According to a second aspect of the present invention there is provided a transgenic starch producing organism comprising exogenous ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme, wherein the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of inorganic phosphate and wherein the activity of the enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

According to a third aspect of the present invention there is provided a potato tuber containing an enhanced starch content.

According to a fourth aspect of the present invention there is provided a method of increasing the rate and/or yield of starch production in an organism, especially a plant or a plant cell, which method comprises introducing into an organism a nucleotide sequence according to the present invention to form a transgenic organism according to the present invention and expressing the nucleotide sequence.

According to a fifth aspect of the present invention there is provided a method of increasing the rate and/or yield of starch production in an organism, especially a plant or a plant cell, which method comprises introducing into or forming in an organism a ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof according to the present invention.

According to a sixth aspect of the present invention there is provided any one of the following: A cDNA sequence identified herein as SEQ ID No. 2, including non-critical allelic variations of that sequence; An amino acid sequence as shown in SEQ ID No. 4, including variants thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence; A cDNA sequence identified herein as SEQ ID No. 5 including non-critical allelic variations of that sequence; An amino acid sequence as shown in SEQ ID No. 6, including variants thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

According to a seventh aspect of the present invention there is provided any one of the following plasmids: Plasmid pPPS1; Plasmid pPPL1; Plasmid pPPL1M; Plasmid pPPS4; Plasmid pPPL4; Plasmid pPPL5; Plasmid pBKL4; Plasmid pVictor IV GN.

According to an eighth aspect of the present invention there is provided a method of increasing the rate and/or yield of starch production in an organism, especially a plant or a plant cell, which method comprises introducing into an organism a recombinant DNA construct containing an exogenous DNA sequence encoding an exogenous ADP glucose pyrophosphorylase enzyme (AGP) or sub-unit thereof and one or more promoter sequences enabling the expression of the AGP encoded by that sequence by the organism thereby to increase the AGP content of the organism and in consequence to increase the rate of starch production by the organism and/or the starch yield, characterised in that the said DNA sequence is the gene sequence encoding the barley (Hordeum vulgare) endosperm AGP or a sub-unit thereof, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units, wherein the construct is expressed in the organism; characterised in that the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of inorganic phosphate; and further characterised in that the activity of the enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

According to a ninth aspect of the present invention there is provided a vector for the transformation of an organism, especially a plant or a plant cell, to increase the AGP content of such an organism consequently to increase the rate of starch production by such an organism, such vector comprising a recombinant DNA construct containing a DNA sequence encoding an exogenous ADP glucose pyrophosphorylase enzyme (AGP), such vector also incorporating the necessary promoter and other sequences enabling the expression of that exogenous AGP in an organism transformed by that vector, characterised in that the said DNA sequence is the gene sequence encoding the barley (Hordeum vulgare) endosperm AGP or a sub-unit thereof, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units, wherein the construct is capable of being expressed in the organism; characterised in that the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of inorganic phosphate; and further characterised in that the activity of the enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

According to a tenth aspect of the present invention there is provided a method of targeting an exogenous protein to the amyloplast of plants or plant cells which comprises introducing into the plant or plant cell a recombinant DNA construct containing a DNA sequence encoding a starch branching enzyme transit peptide and an exogenous DNA sequence encoding the exogenous protein; wherein the construct is capable of being expressed in the plant or plant cells; preferably wherein the DNA sequence encoding the starch branching enzyme comprises the sequence identified as SEQ.ID.No.5 and/or the starch branching enzyme expressed in the plant or plant cell by said construct comprises the amino acid sequence identified as SEQ.ID. No. 6.

According to an eleventh aspect of the present invention there is provided an AGP enzyme or sub-unit thereof whose *in vivo* activity is substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of P_i, and whose activity is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

According to a twelfth aspect of the present invention there is provided a foodstuff made from or comprising an organism according to the present invention; preferably wherein the foodstuff is a fried foodstuff; more preferably wherein the foodstuff is a potato.

The term 'transgenic organism' in relation to the present invention means an organism comprising an expressable exogeneous nucleotide sequence or an expressed product of such an expressable exogeneous nucleotide sequence. Preferably the expressable exogeneous nucleotide sequence is incorporated in the genome of the organism.

The term 'organism' in relation to the present invention includes any starch producing organisms such as plants, algae, fungi and bacteria, as well as cells thereof. Preferably the term means a plant or cell thereof, more preferably a potato and especially a potato tuber.

The term 'nucleotide' in relation to the present invention includes genomic DNA, cDNA, synthetic DNA, and RNA. Preferably it means DNA, more preferably cDNA.

The terms 'allele' and 'variant' in relation to the present invention mean any substitution of, variation of, modification of, replacement of, deletion of or the addition of one or more nucleic acid(s)/amino acids from or to the sequence providing the resultant sequence expresses or exhibits the required enzymatic activity. They also mean a substantial homologous sequence wherein there is homology with respect to at least the essential nucleic acids/nucleic acid residues/amino acids for expression of or exhibition of the required enzymatic activity. Preferably there is at least 80% homology, more preferably at least 90% homology, and even more preferably there is at least 95% homology with the listed sequences. Hybrid sequences are also covered. These may be prepared from at least two different sources - e.g. the variant may include a sequence from one source that gives the variant the independence *vis-a-vis* the level of *in vivo* 3-phospho-glycerate and a sequence from another source that gives the variant the independence *vis-a-vis* the level of *in vivo* P_i.

The term 'sub-unit' in relation to the present invention means an active component of the enzyme that displays the required enzymatic activity. For example, in the case of AGP enzyme obtained from barley (Hordeum vulgare), which is a heterotetramer containing two large sub-units and two small sub-units, the term includes any one of those sub-units as well as combinations thereof as well as a shortened variant thereof.

The term 'retains enzymatic activity' in relation to the present invention means that the activity of the enzyme variant or sub-unit thereof is still substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of inorganic phosphate, but not necessarily to the same extent as the native enzyme.

The term 'substantially independent' in relation to the present invention means that the enzyme has a decreased sensitivity to levels of PGA and/or of inorganic phosphate, preferably at least to PGA. By way of example, in the absence of PGA the levels of AGPase activity of the native enzyme or sub-unit thereof are in the order of at least 0.002 units per mg protein, preferably at least 0.01 units per mg protein - when measured in *Bintje* potato tuber extract. Typically, in the case of the preferred barley AGP enzyme we have found that the AGPase levels of the large sub-unit are greater than the levels of the small sub-unit and are typically in the order of greater than 0.02 units per mg protein and can be in the order of 0.05 units per mg protein - when measured in *Bintje* potato tuber extract. This is in contrast to the known enzymes which have no, or at most negligible, AGPase activity in the absence of PGA.

Preferably the enzymatic activity of the AGP enzyme is at least substantially independent of any level of *in vivo* 3-phospho-glycerate.

More preferably the enzymatic activity of the AGP enzyme is not stimulated by fructose-1,6-bisP and it is not inhibited by AMP.

Preferably the AGP enzyme is a heteromer, preferably a heterotetramer, more preferably a heteromer containing two large sub-units and two small sub-units.

Preferably the AGP enzyme is isolatable from Hordeum, preferably wherein the enzyme is barley (Hordeum vulgare) endosperm AGP or a sub-unit thereof, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units.

Preferably the nucleotide sequence is a DNA sequence.

Preferably the DNA sequence encodes the large sub-unit of the barley endosperm AGP or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequence defining the large sub-unit of the barley endosperm AGP.

Preferably the DNA sequence is the sequence identified herein as SEQ ID No 1, including non-critical allelic variations of that sequence.

Preferably the DNA sequence encodes the small sub-unit of the barley endosperm AGP, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequence defining the small sub-unit of the barley endosperm AGP.

Preferably the DNA sequence is the sequence identified herein as SEQ ID No 2, including non-critical allelic variations of that sequence.

Preferably both of the DNA sequences are expressed in the same organism. The DNA sequences need not be derived from the same initial source, such as barley. It is preferred however that they are from the same source, for example barley.

Preferably, when both of the DNA sequences are expressed in the same organism, each DNA sequence addditionally codes for a different marker - e.g. the large or small sub-unit of barley AGP enzyme may be in a construct that contains a kanamycin resistance gene such as a construct based on plasmid pBKL4 or pVictor IV GN and

another small or large sub-unit f barley AGP enzyme may be in a construct that contains a mannose isomerase gene such as a construct based on plasmid pVictor IV SGiN Man.

Preferably the expressed AGP enzyme or sub-unit thereof comprises the amino acid sequence set out in SEQ ID No. 3, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

Preferably the expressed AGP enzyme or sub-unit thereof comprises the amino acid sequence set out in SEQ ID No. 4, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

Preferably the expressed AGP comprises both a large sub-unit having the amino acid sequence set out in SEQ ID No. 3 or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence, and a small sub-unit having the amino acid sequence set out in SEQ ID No. 4 or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

Preferably the nucleotide sequence additionally codes for a transit peptide which can transport, or assist in the transportation of, the enzyme or sub-unit thereof from the cytoplasm to the relevant or appropriate plastid(s), such as a chloroplast and/or an amyloplast. Preferably the transit peptide is Rubisco Activase transit peptide or Starch Branching enzyme transit peptide.

Preferably the transit peptide is coded for by a DNA sequence comprising the sequence identified as SEQ. I.D. No. 5, including non-critical allelic variations of that sequence.

Preferably the transit peptide has an amino acid sequence comprising the sequence identified as Seq.I.D.No. 6, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at ne or more locations in that sequence.

Preferably the nucleotide sequence is operatively connected to a prom ter which expresses the sequence wherein the promoter is cell, tissue or organ specific.

Preferably the promoter has the sequence identified as SEQ.I.D.No.7, or a variant thereof having non-critical nucleotide substitution(s) or deletion(s) at one or more locations in that sequence.

Preferably the AGP enzyme or sub-unit thereof comprises the amino acid sequence set out in SEQ ID No. 3, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence and/or the amino acid sequence set out in SEQ ID No. 4, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

Preferably the organism is a transgenic plant.

Preferably the transgenic plant is a potato plant.

Preferably the nucleotide sequence according to the present invention is obtainable from any one of the following plasmids: Plasmid pVictor IV SGiN Man; Plasmid pPPS1; Plasmid pPPL1; Plasmid pPPL1M; Plasmid pPPS4; Plasmid pPPL4; Plasmid pPPL5; Plasmid pBKL4; Plasmid pVictor IV GN.

Preferably the enzyme is obtainable from a eukaryotic source.

The present invention has broad applicability to starch producing organisms, especially plants. The present invention works better in organisms such as plants compared to bacteria.

In particular the present invention works better in plants compared to E. Coli where AGP activity is stimulated by fructose-1,6-bisP and inhibited by AMP. This E. Coli pathway is different to the pathway for the biosynthesis of starch in plants and algae.

With regard to one preferred aspect of the present invention, namely a foodstuff prepared from frying a potato according to the present invention, it is to be noted that the increased starch content of the potato will lead to less fat/oil uptake during frying. This results in obvious dietary advantages. Moreover, the increased levels of starch also means that there are decreased free levels of reducing carbohydrates - which are used in starch synthesis - and so there is a decreased tendancy for the resultant product to become discoloured on frying by example reaction of the reducing carbohydrates with the hot fat/oil.

In accordance with a preferred aspect of the present invention it was found that AGP from barley (Hordeum vulgare) endosperm is highly active even in the absence of the activator PGA and is relatively insensitive to PGA/P_i ratios which play an important regulatory function in the case of AGP from most other known plant sources. The PGA/P_i ratio is also believed to play an important regulatory function in non-plant AGP, e.g. algal AGP.

The cDNA sequences encoding parts of the large and the small sub-units of the barley endosperm AGP and the deduced amino acid sequences have recently been established and published in Plant Molecular Biology, 19, 381-389 (1992). The complete DNA sequence encoding the large sub-unit together with the cDNA for the large sub-unit are set out in Plant Physiol. 100, 1617-1618, (1992).

In accordance with the present invention the complete DNA sequence encoding the small sub-unit of the barley endosperm AGP and the deduced amino acid sequence has now been established. Those complete cDNA sequences are reproduced herein as SEQ ID Nos 1 and 2 encoding, respectively, the large and small sub-units of the barley endosperm AGP, whilst the deduced amino acid sequences are set out herein as SEQ ID Nos 3 and 4, respectively.

Thus, in the preferred embodiment of the present invention, it was discovered that starch production in plants can be enhanced/increased by incorporating into the plant's genome and under the control of suitable promoter sequence or sequences

promoting the expression of the gene in the plant cells, particularly in the chloroplasts and amyloplasts, DNA sequences encoding either the large (60 kDa) sub-unit of barley (Hordeum vulgare) endosperm AGP or the small (51 kDa) unit, or both.

Thus, in a highly preferred aspect of the present invention there is provided transgenic plants and plant cells having increased rates of starch production and/or starch content, as compared with the corresponding non-transformed plant or plant cell, such plants and plant cells having been transformed with a recombinant DNA construct containing, in operational relationship (particularly in downstream relationship) to a plant promoter sequence or sequences enabling the expression of the gene in the plant or plant cell, the gene sequence encoding the barley (Hordeum vulgare) endosperm AGP or an active sub-unit thereof retaining the enzymatic activity of the heterotetrameric AGP, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in the amino acid sequences defining the barley endosperm AGP or either of its sub-units.

Whilst, in accordance with the present invention, a wide variety of organisms (e.g. plants and plant cells) may be transformed (especially with the gene encoding barley endosperm AGP or either of its sub-units) to increase starch production and starch yields in that particular organism, the preferred embodiment concerns the transformation of the major starch producing plant crops, namely potato, rice, wheat and maize, which four crops in terms of calorific value, probably account for three quarters of the world's food supply. Sugar beet may also be transformed.

In a more specific aspect of the present invention there are provided transgenic plants and plant cells having increased rates of starch production and/or providing increased starch yields compared with the non-transformed material, such plants and plant cells having been transformed with a recombinant DNA construct containing in downstream relationship to a plant promoter sequence or sequences enabling the expression of the gene in the transformed plant or plant cells, either or both the sequences SEQ ID No 1 and SEQ ID No 2 as set out in the prescribed fashion in the sequence listings annexed hereto and which are taken to be part of the present

specification, or an allelic variant of either sequence showing substantial homology with the listed sequence and containing non-critical nucleotide substitutions at one or more locations in the nucleotide chain.

Alternatively defined, there are provided, in accordance with the present invention, transgenic plants and plant cells showing enhanced levels of AGP production, particularly, in the chloroplasts and amyloplasts, such plants and plant cells having been transformed with a recombinant DNA construct enabling the expression within the plant or plant cells of barley endosperm AGP or either of its sub-units, those sub-units having the derived amino acid sequences set out in SEQ ID Nos. 3 and 4, or a variant of such a sequence having non-critical amino acid substitution(s) or deletion(s) at one or more locations in the amino acid sequence defining the barley endosperm AGP or either of its sub-units.

Also provided in accordance with this invention are plant transformation vectors for the transformation of plants and plant cells to increase the AGP content of such plants and plant cells and thus to increase the rate of production of starch by the transformed plant or plant cell and/or the starch yield, such vectors containing one or more promoter sequences functional in plants linked in operational relationship with a DNA sequence encoding barley endosperm AGP, or either of its sub-units. More especially plant transformation vectors are provided comprising one or more promoter sequences functional in plants linked in operational relationship with either or both the sequences SEQ ID No 1 or SEQ ID No 2, or an allelic variant of either sequence showing substantial (at least 80%) homology with the listed sequence but having non-critical nucleotide substitution(s) at one or more locations in the nucleotide chain.

With regard to the promoter, numerous promoters which are functional in plants are known. The promoter should be capable of allowing suffient expression to result in the desired increase in starch production. Preferably, the promoter should be chosen so that the increased starch production is carried out in the plant tissues where the starch production is required. For instance the promoters of starch biosynthetic genes from plants may be useful.

Known examples of such promoters include the promoter of the granule bound starch synthase gene from potato (Van der Leij et al. [1991] Mol. Gen. Genet. 228: 240-248), and the promoter of the starch branching enzyme gene Sbe 1 from rice (Kawasaki et al. [1993] Mol. Gen. Genet. 237: 1-16).

For expression in potato, a tuber specific class I patatin promoter is preferred (Mignery et al. [1988] Gene. 62: 27-44). The DNA sequence encoding the tuber specific class I patatin promoter is set out in the appendix hereto as SEQ ID No. 7. This patatin promoter was obtained from Dr. William Belknap, USDA - ARS, Alabany, California.

The DNA sequence encoding barley endosperm AGP is preferably linked to other control sequences for the expression of the DNA in addition to a promoter sequence such as a transcription terminator sequence. Transcription terminators may be derived from a variety of different genes, including plant, viral and Agrobacterium genes. A cauliflower mosiac virus 35S terminator is preferred.

AGP activity can occur in different sites in plants. For example in potatoes AGP activity is mainly localised in the chloroplasts (i.e. plastids specialising in photosynthesis) or the amyloplasts (i.e. plastids specialising in starch storage). Many amyloplast-localised proteins are expressed as precursors and are targeted to the amyloplast by an appropriate transit peptide that is subsequnetly removed. Similarly, many chloroplast-localised proteins are expressed as precursors which can be targeted to the chloroplast by an appropriate target peptide.

Whilst not wishing to be bound by any theory, it is believed that both the large and small sub-units of the barley endosperm AGP are synthesised as precursor peptides. Additional sequences are found to be attached to the amino-termini of the mature proteins which are understood to represent transit peptides. The transit peptide is then cleaved upon sequestration of the presursor protein into the plastid. It is understood that the enzyme is not subjected to any other post-translation modification process *in vivo*.

However, in another embodiment of the present invention, it is desirable for the AGP transit peptides to be supplemented with one or more additional transit peptides. The transit peptide can be conveniently fused directly at the amino terminal methionine of the AGP barley sub-unit. In further preferred embodiments the barley AGP transit peptide can be substituted by another amyloplast or chloroplast transit peptide. The barley endosperm AGP cDNA is inserted into a convenient cloning vector, e.g. a plasmid, at a suitable restriction site. The DNA sequence of interest can be encloned into further vectors, if necessary, for the incorporation of additional DNA sequences. Suitable plant transit peptides include known chloroplast (Gavel & Von Heine [1990] FEBS Lett. 261: 455-458) or amyloplast (Van der Leij et al. [1991] Mol. Genet. 228: 240-248; Klosgen et al. [1989] Mol. Gen. Genet. 217: 155-161; Brisson et al. The Plant Cell [1989] 1: 559-566) transit peptides.

In potatoes, preferably a rubisco activase transit peptide (Werneke et al. Proc. Natl. Sci. USA [1988] 85: 787-791) or a starch branching enzyme transit peptide is used. The 480 bp starch branching enzyme cDNA sequence from potato showing 120 nucleotides of the 5' untranslated region and 360 nucleotides of the coding region (see SEQ.I.D. No. 5), which contains a putative 75 amino acid transit peptide and 45 amino acids of the mature branching enzyme is set out in the appendix hereto as SEQ ID No. 6.

In addition to the transit peptide portion of a protein, it may be desirable to include sequences encoding a portion of the mature plastid-targeted protein to further facilitate intracellular transport.

Preferably the plasmids are also provided with selection markers to enable the transformed plant cells to be separated out from plant cells which have not been transformed. Suitable genes are known and include e.g. a neomycin phosphotransferase gene (e.g. neo npt II), a phosphinotricine/bialaphos acetyl-tranferase gene (e.g. bar) and a ß-glucuronidase gene (e.g. uidA) or a phosphomannose isomerase gene (e.g. manA, pmi).

In a preferred embodiment, the transformation vectors may be prepared by initially obtaining cDNA encoding the small and large units f barley endosperm AGP by the method described in Plant Molecular Biology, 19, 381-389 (1992). For ligation into a convenient cloning vector, e.g. a plasmid, the barley endosperm AGP cDNA is provided with restriction sites at each end by PCR using the oligonucleotide primers obtained by conventional oligonucleotide synthesis procedures or a commercially available oligonucleotide synthesizer such as, for example, Applied Biosystems 381 A DNA synthesizer. These restriction sites should be homologous with sequences in the cloning vector. The desired DNA sequence can be recloned into further vectors for preparation of the ultimate transformation vectors for preparing the transgenic starch producing organism, especially a transgenic plant.

In the preferred embodiment of the present invention, the plant or plant cells may be transformed by any suitable technique for transforming cells - such as use of T-DNA, electroporation, injection, DNA bombardment or fusion. After transformation, a whole plant can be cultivated from a transformed plant cell in the usual manner.

Preferably, transformation of the plant cell is achieved with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. If agrobacteria are used for transformation, the barley endosperm cDNA needs to be incorporated initially into either an intermediate vector or a binary vector. The intermediate vectors can be integrated into *Agrobacterium tumefaciens* by means of a helper plasmid. Preferably binary vectors are used, which can be transformed directly into agrobacteria. Binary vectors comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. The agrobacteria used as host cell should comprise a plasmid carrying a vir region, which is necessary for the transfer of the T-DNA into the plant cell. Transformation using Agrobacterium is achieved by cultivating the Agrobacterium with the plant cell.

Depending on the plant species to be transformed, a variety of different plant transformation vectors can be used. These include pBIN 121, pAL4404, pEHA101, pBKL4, pVictor IV SGiNMan and pVictor IV GN.

For the transformation of potato species Agrobacteria the preferred plant transformation vectors are the plasmids pBKL4, pVictor IV SGiNMan and pVictor IV GN. These plasmids are described later in greater detail.

Preferred plasmids used in the construction of the plasmid used for transformations include pPATA1 and pBluescript II KS. Plasmid pBluescript II KS is a widely used cloning vector available from Stratagene.

Plants can be confirmed as transformed by performing conventional blotting assays and PCR.

The starch content of the plants can be analysed based upon the specific gravity determined using the weight in water and the weight in air as described by W.A. Gould In: Chipping Potato Handbook, ed. Gould, W.A. The Snack Food Association, Vermont, 1989, pp 18-22, in an article entitled "Specific gravity, its measurement and use.

The limitation of the exogenous ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme being not stimulated by fructose-1,6-bisP and/or not inhibited by AMP, which further distinguishes the present invention from the AGP enzymes of the prior art such as those of WO 91/19806 and WO 92/11382, can be expressed in the alternative as either the exogenous ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme not being only just an *E. Coli* AGP enzyme, or the exogenous ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme being capable of catalysing the reaction

$$\alpha\text{-glucose-1-P} + \text{ATP} \longleftrightarrow \text{ADP-glucose} + \text{PP}_i \,.$$

The following samples were deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St. Machar Drive, Aberdeen, Scotland. United Kingdom, AB2 1RY on 29 March 1994:

E. Coli containing plasmid pPPS1 (NCIMB 40618);

E. Coli containing plasmid pPPL1 (NCIMB 40619);

E. Coli containing plasmid pPPS4 (NCIMB 40620);

E. Coli containing plasmid pPPL4 (NCIMB 40621); and

E. Coli containing plasmid pPPL5 (NCIMB 40622).

The following samples were deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St. Machar Drive, Aberdeen, Scotland, United Kingdom, AB2 1RY on 31 March 1994:

E. Coli containing plasmid pBKL4 (NCIMB 40623);

E. Coli containing plasmid pVictor IV GN (NCIMB 40624); and

E. Coli containing plasmid pVictor IV SGiN Man (NCIMB 40625).

A detailed construction of plant transformation vectors according to the present invention and the transformation of plants and plant cells using those vectors to produce transgenic plants according to this invention having increased rates of starch biosynthesis and/or starch yield will now be described in more detail

In this regard, the present invention will now be described only by way of example.

In the following Examples reference is made to the accompanying figures in which:

Figure 1 shows the restriction map for plasmid pPATA1;
Figure 2 shows the restriction map for plant transformation vector pBKL4;
Figure 3 shows the restriction map for plasmid pPPS1;
Figure 4 shows the restriction map for plasmid pPPL1;
Figure 5 shows the restriction map for plasmid pATP1;
Figure 6 shows the restriction map for plant transformation vector pVictor IV GN;
Figure 7 shows the restriction map for plant transformation vector pVictor IV SGin Man;
Figure 8 shows the N terminal amino acid sequence of the rubisco activase - AGP small subunit fusion enzyme, the N terminal amino acid sequence of the rubisco activase - AGP large subunit fusion enzyme and the N terminal amino acid sequence of the starch branching enzyme - AGP large subunit fusion enzyme;
Figure 9 shows the restriction map for plasmid pPPS4;
Figure 10 shows the restriction map for plasmid pBETP5;
Figure 11 shows the restriction map for plasmid pPPL4;
Figure 12 shows the restriction map for plasmid pPPL5;
Figure 13 shows the restriction map for plasmid pPPL1M;
Figure 14 shows the cDNA and amino acid sequences for the large sub-unit of barley AGP;
Figure 15 shows the cDNA and amino acid sequences for the small sub-unit of barley AGP;
Figure 16 shows the cDNA (first 480 nucleotides from 5' end) and amino acid (first 120 amino acids from amino terminus) sequences for the starch branching enzyme; and
Figure 17 shows the genomic DNA sequence for the preferred potato promoter.

In the following Examples the foll wing amino acid codes are used:

Symbol	3-letter	Meaning
A	Ala	Alanine
B	Asp, Asn	Aspartic Asparagine
C	Cys	Cysteine
D	Asp	Aspartic
E	Glu	Glutamic
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
X	Xxx	Unknown
T	Tyr	Tyrosine
Z	Glu, Gln	Glutamic Glutamine
+	End	Terminator

A. **Construction of plant transformation vectors containing the ADP-glucose pyrophosphorylase genes expressed from a patatin class I promoter**

EXAMPLE 1

Plasmid pPPS1

Plasmid pPPS1 is a pBKL4 derivative containing the construction:

Patatin promoter - small sub-unit AGP cDNA - 35S terminator

The AGP cassette was inserted in the KpnI site of pBKL4. The additional elements introduced in the pBKL4 T-DNA by this insertion are descibed below.

Patatin promoter: The patatin promoter is a tuber specific promoter from potato (Mignery et al. 1988, Gene 62:27-44) - see SEQ. ID. No. 7.

Small subunit ADP-glucose pyrophosphorylase (bepsF2): This is a 1.8 kb cDNA fragment encoding the small subunit ADP-glucose pyrophosphorylase from barley endosperm - see SEQ ID. No. 2.

35S terminator: The CaMV 35S terminator (Odell et al. 1985, Nature 313:810-812) is fused to the bepsF2 fragment.

In more detail, a 1.8 kb BamHI cDNA fragment encoding the barley endosperm ADP glucose pyrophosphorylase small subunit (beps) was cloned in the BamHI site of plasmid pPATA 1 (Figure 1). Plasmid pPATA 1 is a derivative of plasmid pUC19 and has tuber specific patatin class I promoter ID SEQ No. 7, a polylinker cloning region, and a 35S terminator. From the resulting plasmid the 3.1 kb KpnI fragment containing the patatin promoter, the beps cDNA and the 35S terminator was isolated and inserted in the KpnI site of the plant transformation vector pBKL4 (Figure 2) to yield plasmid pPPS1 (Figure 3).

Plasmid pBKL4 is a derivative of plasmid pBIN19 for Agrobacterium tumefaciens mediated transformation of plants and harbours a T-DNA region with a β-glucuronidase gene (GUS) transcribed from a 35S promoter and terminated at the nopaline synthase gene terminator, a polylinker cloning region, and a neophosphotransferase gene transcribed from a 35S promoter and terminated at the octopine synthase gene terminator.

EXAMPLE 2

Plasmid pPPL1

Plasmid pPPL1 is a pBKL4 derivative containing the construction:

Patatin promoter - Large subunit ADP-glucose pyrophosphorylase cDNA - 35S terminator.

The ADP-glucose pyrophophorylase cassette was inserted in the EcoRI site of pBKL4.

The additional elements introduced in the pBKL4 T-DNA by this insertion are described below.

Patatin promoter: The patatin promoter is a tuber specific promoter from potato (Mignery et al. 1988, Gene 62:27-44) - see SEQ. ID. No. 7.

Large subunit ADP-glucose pyrophosphorylase (bep110): This is a 1.9 kb cDNA fragment encoding the large subunit ADP-glucose pyrophosphorylase from barley endosperm (Villand et al. 1992, Plant Physiol 100:1617-1618) - see SEQ ID. No. 1.

35S terminator: The CaMV 35S terminator (Odell et al. 1985, Nature 313:810-812) is fused to the bepl 10 fragment.

In more detail, a 1.9 kb EcoRI-HindIII cDNA fragment encoding the barley endosperm ADP glucose pyrophosphorylase large subunit (bepl) was isolated, the restriction ends were filled in with klenow DNA polymerase, and the blunt ended DNA fragment was cloned in the SmaI site of plasmid pPATA1. From the resulting plasmid the 3.2 kb EcoRI fragment containing the patatin promoter, the bepl cDNA, and the 35S terminator was isolated and inserted in the EcoRI site of the plant transformation vector pBKL4 to yield plasmid pPPL1 (Figure 4).

EXAMPLE 3

Plasmid pPPL1M

Plasmid pPPL1M (see Figure 13) is similar to pPPL1 except that the ADP-glucose pyrophosphorylase cassette:

Patatin promoter - Large subunit ADP-glucose pyrophosphorylase - 35S terminator.

was inserted in the EcoRI site of pVictorIV SGiN Man.

EXAMPLE 4

Plasmid pVictor IV SGN

pVictorIV SGN (Figure 6) is a vector for *Agrobacterium* mediated plant transformation, and contains the Ti right and left border sequences from the nopaline type pTiT37 plasmid (Yadav et al. 1982, Proc Natl Acad Sci 79:6322-6326) flanking the genes encoding kanamycin resistance (NPTII) and ß-glucoronidase (GUS).

For replication and maintenance in *E. coli* the plasmid contains the origin of replication from the *E. coli* plasmid pUC19 (pUC19ori) Yanish-Perron et al. 1985 Gene 33:103-119), and for replication and maintenance in *Agrobacterium tumefaciens* the plasmid further contains the origin of replication from the *Pseudomonas* plasmid

pVS1 (pVS1ori) (Itoh et al. 1984, Plasmid 11:206-220, It h and Haas 1985, Gene 36:27-36). For selection in *E. coli* and *Agrobacterium tumefaciens* the plasmid contains the spectinomycin/streptomycin resistance gene (spec.strep) from the transposon Tn7 encoding the enzyme 3'(9)-O- nucleotidyltransferase (Fling et al. 1985, Nucleic Acids Res 19:7095-7106). The spec/strep resistance gene is fused to the *tac* promoter for efficient expression in the bacterium.

The T-DNA segment between the right and left border harbours the following genes, which are the only genes transferred to the potato plant via the *Agrobacterium tumefaciens* mediated transformation.

ß-glucuronidase (GUS): This segment next to the right border is the ß-glucuronidase gene (GUS) from *E. coli* (Jefferson et al., 1986, Proc Natl Acad Sci 83:8447-8451) fused to the CaMV 35S promoter (35S) and 35S terminator (35St) (Odell et al. 1985, Nature 313:810-812).

Multiple cloning sites (MCS): A polylinker containing various restriction endonuclease recognition sites is inserted after the 35S terminator.

Kanamycin resistance (NPTII): The segment next to the MCS is the kanamycin (neomycin) phosphotransferase gene (NPTII) from the transposon Tn5 (Beck et al. 1982 Gene 19:327-336) fused to the CaMV 35S promoter (Odell et al. 1985, Nature 313:810-812) and the terminator of the octopine synthase gene (Caplan et al. 1983, Science 222:815-821).

EXAMPLE 5

Plasmid pVictor IV SGiN Man

pVictorIV SGiN Man (Figure 7) is similar to pVictorIV SGN (Bilag XI) except that the GUS gene is replaced by another GUS gene containing an intron (GUSintron) to prevent expression in bacteria.

Moreover, the kanamycin (neomycin) phosphotransferase gene (NPTII) has been replaced by the mannose-6-phosphate isomerases gene, *man*A, from *E. coli*.

ß-glucuronidase (GUSintron): This segment next to the right border is the ß-glucuronidase gene (GUS) from *E. coli* (Jefferson et al. 1986, Proc Natl Acad Sci 83:8447-8451) furnished with an intron to prevent expression in bacteria, and fused to the CaMV 35S promoter (35S) and 35S terminator (35St) (Odell et al. 1985, Nature 313:810-812).

Mannose-6-phosphate isomerase: This segment is the mannose-6-phosphate isomerases gene, *man*A, from *E. coli* (Miles and Guest 1984, Gene 32:41-48) fused to the enhanced 35S promoter (E35S) (Kay et al. 1987, Science 236:1299-1302) and 35S terminator (35St) (Odell et al. 1985, Nature 313:810-812). The phosphomannose isomerase gene is used as a selection marker to select transgenic shoots on a media containing D-mannose as the carbon source.

B. Attachment of transit peptides to the ADP-Glucose pyrophosphorylase subunits

EXAMPLE 6

Plasmid pPPS4

pPPS4 (Figure 9) is a pVictorIV derivative in which a 3 kb KpnI fragment containing the construct

Patatin promoter - spinach rubisco activase transit peptide - small subunit AGP from barley endosperm - 35S terminator

is inserted in the KpnI site.

In more detail, the coding region of the barley end sperm ADP glucose pyrophosphorylase small subunit (beps) cDNA was amplified by PCR using the primers:

5' CGG GAT CCA TGG ATG TAC CTT TGG CA 3'

and

5' CGG GAT CCT TAT TTA TTT ATA TGA CTG TTC CAC TAG 3'

which provide the PCR fragment with a BamHI and a NcoI in the 5' end and a BamHI site at the 3' end. The 1.4 kb BamHI fragment containing the entire coding region of the AGP small subunit plus two additional amino acids (G and S) at the amino-terminal end was cloned in the BamHI site of pBluescript II KS to yield plasmid pBBSF. The 1.4 kb BamHI fragment was isolated from pBBSF and cloned in the BamHI site of plasmid pATP1 (Figure 5). Plasmid pATP1 has a patatin promoter, a 58 amino acid rubisco activase transit peptide DNA and 35 amino acids of the mature enzyme, a BamHI site that facilitates in frame fusion of the small subunit AGP reading frame, and a 35S terminator. The 3 kb KpnI fragment including the patatin promoter, the activase transit peptide, the AGP coding region, and the 35S terminator was isolated from the resulting plasmid and cloned in the KpnI site of the plant transformation vector pVictorIV GN (Figure 6) to give plasmid pPPS4 (Figure 9).

Amino terminal amino acid sequence of the rubisco activase - AGP small subunit fusion enzyme.

1 MATAVSTVGA ATRAPLNLNG SSAGASVPTS GFLGSSLKKH 40

41 TNVRFPSSSR TTSMTVKAAE NEEKNTDKWA HLAKDFSDDQ 80

81 LDIRRGKGMV DSLGSMDVPL ASKVPLPSPS KHEQCNVYSH 120

The rubisco activase sequences starts at amino acid residue 1 and ends at leucine residue at 93, while the AGP small subunit sequences begins with the methionine at 96. The rubisco activase transit peptide is cleaved at the alanine residue at 58 leaving the alanine at 59 as the N-terminal amino acid.

The above sequence is listed later on as SEQ.I.D. No. 8.

EXAMPLE 7

Plasmid pPPL4

pPPL4 is a pVictorIV SGiN Man derivative in which a 3.2 kb EcoRI fragment containing the construct

Patatin promoter - spinach rubisco activase transit peptide - large subunit AGP from barley endosperm - 35 S terminator

is inserted in the EcoRI site.

In more detail, the coding region of the barley endosperm ADP glucose pyrophosphorylase large subunit (bepl) cDNA was amplified by PCR using the primers

5' GCG GAT CCA TAT CGA GTT CAG CGT 3'
and
5' CGG GAT CCG CAC AGG TTG TCG CAG AAC 3'

which provide the PCR fragment with a BamHI and a NdeI in the 5' end and a BamHI site at the 3' end. The 1.6 kb BamHI fragment containing the entire coding region of the AGP large subunit plus two additional amino acids (I and H) at the amino-terminal end was cloned in the BamHI site of pBluescript II KS to yield plasmid pBBLF.

The 1.6 kb BamHI fragment was isolated from pBBLF and cloned in the BamHI site of plasmid pATP2. Plasmid pATP2 has a patatin promoter, a 58 amino acid rubisco activase transit peptide DNA and 35 amino acids of the mature enzyme, a BamHI site that facilitates in frame fusion of the large subunit AGP reading frame, and a 35S terminator. The 3 kb EcoRI fragment including the patatin promoter, the activase transit peptide, the AGP coding region, and the 35S terminator was isolated from the resulting plasmid and cloned in the EcoRI site of the plant transformation vector pVictor IV SGiN Man (Figure 7) to form plasmid pPPL4 (Figure 11).

Amino terminal amino acid sequence of the rubisco activase - AGP large subunit fusion enzyme.

1	MATAVSTVGA ATRAPLNLNG SSAGASVPTS GFLGSSLKKH	40
41	TNVRFPSSSR TTSMTVKAAE NEEKNTDKWA HLAKDFSDDQ	80
81	LDIRRGKGMV DSLGIHMQFS SVLPLEGKAC VSPVRREGSA	120

The rubisco activase sequences starts at amino acid residue 1 and ends at the leucine residue at 93, while the AGP large subunit sequences begins with the methionine at 97. The rubisco activase transit peptide is cleaved at the alanine residue at 58 leaving the alanine at 59 as the N-terminal amino acid. The above sequence is listed later on as SEQ.I.D. No. 9.

EXAMPLE 8

Plasmid pPPL5

pPPL5 is a pVictorIV GIN MAN derivative in which a 3.4 kb EcoRI fragment containing the construct

Patatin promoter - potato starch branching enzyme amyloplast transit peptide - large subunit AGP from barley endosperm - 35S terminator

is inserted in the EcoRI site.

The coding region of the barley endosperm ADP glucose pyrophosphorylase large subunit was amplified by PCR using the primers:

5' CGG GAT CCG ATG CAG TTC AGC AGC GTG 3'

and

5' CGG GAT CCG CAC AGG TTG TCG CAG AAC 3'

which provide a 1.62 kb PCR fragment with BamHI ends. The BamHI fragment containing the entire coding region of the AGP large subunit plus one additional amino acid (P) at the amino terminal end was inserted in the BamHI site of pBETP5 (Figure 10). In this way the AGP large subunit was fused to the 75 amino acid potato starch branching enzyme transit peptide plus 26 amino acids of the mature branching enzyme. The fusion enzyme is expressed from a patatin promoter and terminated at a 35S terminator. The 3.4 Kb EcoRI fragment from the resulting plasmid (pPBL1) containing the patatin promoter, the starch branching enzyme transit peptide-AGP large subunit fusion enzyme, and the 35S terminator, was inserted in the EcoRI site of the plant transformation vector pVictorIV SGiN Man yielding plasmid pPPL5 (Figure 12).

Amino terminal amino acid sequence of the starch branching enzyme - AGP large subunit fusion enzyme.

1	MEINFKVLSK PIRGSFPSFS PKVSSGASRN KICFPSQHST	40
41	GLKFGSQERS WDISSTPKSR VRKDERMKHS SAISAVLTDD	80
81	NSTMAPLEED VKTENIGLLN LDPMQFSSVL PLEGKACVSP	120

The starch branching enzyme sequences starts at amino acid residue 1 and ends at 103, while the AGP large subunit sequence begins with the methionine at 104.The starch branching enzyme transit peptide is cleaved at the alanine residue (75) leaving the valine residue (76) as the amino terminal amino acid.

The above sequence is listed later on as SEQ.I.D. No. 10.

C. Production of transgenic potato plants containing the AGP-gene

EXAMPLE 9

Axenic stock cultures

Shoot cultures of Solanum tuberosum 'Bintje' and 'Dianella' are maintained on a substrate (LS) of a formula according to Linsmaier, E.U. and Skoog, F. (1965), Physiol. Plant. 18: 100-127, in addition containing 2 μM silver thiosulphate at 25°C and 16 h light/8 h dark.

The cultures are subcultured after approximately 40 days. Leaves are cut off the shoots and cut into nodal segments (approximately 0.8 cm) each containing one node.

Inoculation of potato tissues

Shoots from approximately 40 days old shoot cultures (height approximately 5-6 cms) were cut into internodal segments (approximately 0.8 cm). The segments are placed into liquid LS-substrate containing the transformed Agrobacterium tumefaciens containing the binary vector of interest. The Agrobacterium are grown overnight in YMB-substrate (dipotassiumhydrogen phosphate, trihydrate (0.66 g/l); magnesium sulphate, heptahydrate (0.20 g/l); sodium chloride (0.10 g/l); mannitol (10.0 g/l); and yeast extract (0.40 g/l)) containing appropriate antibiotics (corresponding to the resistance gene of the Agrobacterium strain) to an optical density at 660 nm (OD-660) of approximately 0.8, centrifuged and resuspended in the LS-substrate to an OD-660 of 0.5.

The segments are left in the suspension of Agrobacterium for 30 minutes and then the excess of bacteria are removed by blotting the segments on sterile filter paper.

Co-cultivation

The shoot segments are co-cultured with bacteria for 48 hours directly on LS-substrate containing agar (8.0 g/l), 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and trans-zeatin (0.5 mg/l). The substrate and also the explants are covered with sterile filter papers, and the petri dishes are placed at 25°C and 16 h light/ 8 dark.

"Washing" procedure

After the 48 h on the co-cultivation substrate the segments are transferred to containers containing liquid LS-substrate containing 800 mg/l carbenicillin. The containers are gently shaken and by this procedure the major part of the Agrobacterium are washed off the segments and/or killed.

Selection

After the washing procedure the segments are transferred to plates containing the LS-substrate, agar (8 g/l), trans-zeatin (1-5 mg/l), gibberellic acid (0.1 mg/l), carbenicillin (800 mg/l), and kanamycin sulphate (50-100 mg/l) or phosphinotricin (1-5 mg/l) or mannose (5 g/l) depending on the vector construction used.

The segments are sub-cultured to fresh substrate each 3-4 weeks.

In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continues for 3-4 months.

Rooting of regenerated shoots

The regenerated shoots are transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

- The transgenic genotype f the regenerated shoot are verified by testing the rooting ability on the above mentioned substrates containing kanamycin sulphate (200 mg/l), by performing NPTII assays (Radke, S. E. et al, Theor. Appl. Genet. (1988), 75: 685-694) or by performing a GUS assay on the co-introduced β-glucuronidase gene according to Hodal, L. et al. Pl. Sci. (1992), 87: 115-122 or by assaying the for the expression of the barley AGP mRNA or AGP enzyme activity as described elsewhere. Plants which are not positive in any of these assays are discarded or used as controls.

Transfer to soil

The newly rooted plants (height approx. 2-3 cms) are transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m²/sec).

When the plants are well established they are transferred to the greenhouse, where they are grown until tubers have developed and the upper part of the plants are senescing.

Harvesting

The potatoes were harvested after about 3 months.

AGP Assay

Tubers from the harvested potato plants were stored at 4°C. AGPase was extracted by homogenization of 10-20g of thinly sliced potato tubers in 20 ml of buffer containing 25 mM Hepes (pH 7.4) mM mercaptoethanol and 1 mM DTT. Homogenization was performed at 0-4°C using 30 ml Waring blender at full speed for 15 seconds. Aliquots of crude extract were then immediately centrifuged at maximal speed for 1 min using bench Eppendorf microcentrifuge and then assayed f r AGPase activity. Assays were carried out immediately after centrifugation to make sure that the enzyme will not be inactivated during storage.

Assays were carried out in the pyrophosphorolysis direction monitoring glucose-1-P formation at 340 nm (21°C), using LKB spectrophotometer (Ultrospec II). Assay mixtures (1 ml) contained: 100 mM Mops (pH 7.4), 0.6 mM NAD, 7mM $MgCl_2$, 1 mM ADP-glucose, 1 mM inorganic pyrophosphate, 10 uM glucose-1,6-biphosphate, 2 units each of glucose-6-P dehydrogenase and phosphoglucomutase. In some instances, 2 mM 3-phosphoglyceric acid (PGA) was added to assays. Assays were run (-PGA) for ca. 5 min, and then PGA was added and assays were monitored for another 5-10 min. Rates were usually linear during the time-course of assays. One unit of AGPase activity corresponds to the amount of enzyme producing 1 umole of NADH under assay conditions.

Starch Analysis

The starch contents of potato tubers was determined according to a method which was designed and proposed by the Dutch-German working group "Standardization" and published in "Methods of assessment for potatoes and potato products". The method was developed for use with a sample size of 5000 g but we scaled the method down for use with potato tubers from a single plant, usually between 20 and 200 g.

All potatoes from a plant are washed and dried with a cloth before weighing (a grams) on an electronic balance. Later, the tubers are weighed again, but this time on a balance with two metal baskets of which one is immersed in a water basin. The potato tubers are placed in the bucket in water, and their weight (b grams) in water is determined.

The under-water weight of a sample is calculated at 5000*b/a grams. From tables showing the relation between under-water weight, dry matter and starch content, the two latter figures can be determined.

This procedure is described in more detail by W.A. Gould in Chipping Potato Handbook, ed. Gould, W.A. The Snack Food Association, Vermont, 1989, pp 18-22, in an article entitled "Specific gravity, its measurement and use.

RESULTS

AGPase levels and starch levels were increased with the constructs of the present invention, particularly in the absence of PGA, especially with the constructs coding for the large sub-unit and in particular the constructs coding for a transit peptide.

In this regard, some results are shown in Table I (below) for transformed potatoes comprising constructs derived from plasmids pPPS4 and pPPL4.

TABLE I

STARCH CONTENT IN TRANSFORMED POTATOES (TRANS) COMPARED TO CONTROL NATIVE POTATOES GROWN UNDER THE SAME CONDITIONS

Sample	% Overall Starch Content	%Starch Content vis-a-vis Control
Control	16	100
Trans 1	19	119
Trans 2	23	144
Trans 3	26	163
Trans 4	19	119
Trans 5	26	163
Trans 6	24	150
Trans 7	21	131
Trans 8	17	106
Trans 9	21	131

The above results clearly show that the average starch level in the transgenic potatoes comprising constructs derived from plasmids pPPS4 and pPPL4 are increased to about 136 % of that found in native potatoes.

Furthermore, the average starch level for the upper two quartiles for the transgenic potatoes comprising constructs derived from plasmids pPPS4 and pPPL4 is about 155 % of that found in native potatoes.

Each of these findings is significant.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION

NAME OF APPLICANTS: DANISCO A/S
BUSINESS ADDRESS: Langebrogade 1
DK-1001 Copenhagen K
Denmark

TITLE OF INVENTION: TRANSGENIC ORGANISM

(2a) INFORMATION FOR SEQUENCE I.D. 1

SEQUENCE TYPE: NUCLEIC ACID
MOLECULE TYPE: DNA
ORIGINAL SOURCE: BARLEY
SEQUENCE LENGTH: 2037
STRANDEDNESS: DOUBLE
TOPOLOGY: LINEAR
SEQUENCE: Nucleotide sequence of a cDNA encoding the large subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (bepl10)

SEQ. ID. No. 1

1 ACGACCACCT CCGAACTCAA CGCCTCCACG GACCATCTCT
41 CTCCTCTCCC CTCCCCTCAC CACCACCACC ACCACCACCC
81 CTTCTCCCTC CCTGCATTTG ATTCGTTCAT ATTCATCCGT
121 CGCTTGCCCG GTCGCCACCC CGTCGATCCC TCACCCCGCC
161 GTCCCCGGCA GTTGCAGGTG GACTGCTAAT GTCATCGATG
201 CAGTTCAGCA GCGTGCTGCC CCTGGAGGGC AAGGCGTGCG
241 TTTCCCCAGT CAGGAGAGAG GGATCGGCCT GCGAGCGCCT
281 CAAGATCGGG GACAGCAGCA GCATCAGGCA CGAGAGAGCG
321 TCCAGGAGGA TGTGCAACGG CGGCGCAGGG GCCCCGCCGC
361 CACCGGTGCG CAGTGCGTGC TCACCTCCGA CGCCAGCCCG
401 GCCGACACCC TTGTTCTCCG GACGTCCTTC CGGAGGAATT
ACGCCGATCC GAACGAGGTC GCGGCCGTCG GTCGCGGCCG
TCATACTCGG CGGCGGCACC GGGACTCAGC TCTTCCCGCT
CACAAGCACA AGGGCCACAC CTGCTGTTCC TATTGGAGGA
TGTTACAGGC TCATCGATAT TCCCATGAGC AACTGCTTCA
601 ACAGTGGCAT CAACAAGATA TTCGTCATGA CCCAGTTCAA
CTCGGCATCT CTCAATCGCC ACATTCACCG CACCTACCTC
GGCGGGGGAA TCAATTTCAC TGATGGATCT GTTGAGGTAT
TGGCCGCGAC ACAAATGCCT GGGGAGGCTG CTGGATGGTT
CCGCGGAACA GCGGATGCCG TCAGAAAATT TATCTGGGTG
801 CTTGAGGACT ACTATAAGCA TAAATCCATA GAGCACATTT
TGATCTTGTC GGGCGATCAG CTTTATCGCA TGGATTACAT

GGAGCTTGTG CAGAAACATG TGGATGACAA TGCTGACATT
ACTTTATCAT GTGCCCCTGT TGGAGAGAGC CGGGCATCTG
AGTACGGGCT AGTGAAGTTC GACAGTTCAG GCCGTGTGAT
1001 CCAGTTTTCT GAGAAGCCAA AGGGCGACGA TCTGGAAGCG
ATGAAAGTGG ATACCAGTTT TCTCAATTTC GCCATAGACG
ACCCTGCTAA ATATCCATAC ATTGCTTCGA TGGGAGTTTA
TGTCTTCAAG AGAGATGTTC TGCTGAACCT TCTAAAGTCA
AGATACGCAG AACTACATGA CTTTGGGTCT GAAATCCTCC
1201 CGAGAGCTCT GCATGATCAC AATGTACAGG CATATGTCTT
CACTGACTAC TGGGAGGACA TTGGAACAAT CAGATCCTTC
TTCGATGCGA ACATGGCCCT CTGCGAACAG CCTCCAAAGT
TTGAATTTTA TGATCCAAAA ACCCCCTTCT TCACTTCGCC
TCGGTACTTA CCGCCAACAA AGTCAGACAA GTGCAGGATC
1401 AAAGAAGCGA TCATTTCGCA CGGCTGCTTC TTGCGTGAAT
GCAAAATCGA GCACTCCATC ATCGGCGTTC GTTCACGCCT
AAACTCCGGA AGCGAGCTCA AGAACGCGAT GATGATGGGC
GCGGACTCGT ACGAGACCGA GGACGAGATC TCGAGGCTGA
TGTCTGAGGG CAAGGTTCCC ATCGGCGTCG GGGAGAACAC
1601 AAAGATCAGC AACTGCATCA TCGACATGAA CGCGAGGATA
GGAAGGGACG TGGTCATCTC AAACAAGGAG GGGGTGCAAG
AAGCCGACAG GCCGGAGGAA GGGTACTACA TCAGGTCCGG
GATCGTGGTG ATCCAGAAGA ACGCGACCAT CAAGGACGGC
ACCGTCGTGT AGGGCGTGCC GGGTCGGCGC GACGGGGTTC
1801 TGCGACAACC TGTGCGCTGC GTCGGTCGTC ATCATCTTCT
CAAACTCCGG GACTGAAGAA GTGATCCGGG GACGGGAGAC
GTTTGAAGCT TGAATGACTG AGACTGAAAG TGAAGGCGCA
GCAGAGGCAG GCAGCATTAG TAGTAAGTAG TAAGTAAGTA
GCAGTGGAAC AAAGTAATAG TCGTTCGTTT TTCCCCTGTA
2001 ATAAATAAGA GGCTGTGTGT TGAGGTAAAA AAAAAAA

In the event that this sequence contains an error, see the corresponding sequence in the accompanying figures.

(2b) INFORMATION FOR SEQUENCE I.D. 2

SEQUENCE TYPE:	NUCLEIC ACID
MOLECULE TYPE:	DNA
ORIGINAL SOURCE:	BARLEY
SEQUENCE LENGTH:	1822
STRANDEDNESS:	DOUBLE
TOPOLOGY:	LINEAR
SEQUENCE:	Nucleotide sequence of a cDNA encoding the small subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (beps)
COMMENT:	The "." at 1569 denotes a purine.

SEQ. ID. No. 2

```
1     AAAAGTGAAC TCACACATCA CTCAATATCT ATATCCTTCC
      ATTTTATATC CCTCGGTGAT GGATGTACCT TTGGCATCTA
      AAGTTCCCTT GCCCTCCCCT TCCAAGCATG AACAATGCAA
      CGTTTATAGT CATAAGAGCT CATCGAAGCA TGCAGATCTC
      AATCCCCATG CTATTGATAG TGTTCTCGGT ATCATTCTTG
201   GAGGTGGTGC AGGGACTAGA TTGTATCCCC TGACGAAGAA
      GCGTGCAAAG CCTGCAGTGC CATTGGGTGC CAACTACAGG
      CTTATTGATA TTCCTGTCAG TAATTGTCTG AACAGCAACA
      TATCAAAGAT CTATGTGCTT ACACAGTTCA ACTCAGCTTC
      TCTTAATCGT CATCTCTCAC GAGCCTATGG GAGCAACATT
401   GGAGGTTACA AGAATGAAGG ATTTGTTGAA GTCCTTGCTG
      CACAGCAGAG CCCAGATAAC CCTGACTGGT TCCAGGGTAC
      TGCAGATGCT GTAAGGCAGT ACTTGTGGCT ATTCGAGGAG
      CATAATGTTA TGGAGTATCT AATTCTTGCT GGAGATCACC
      TGTACCGAAT GGACTATGAA AAGTTTATTC AGGCACACAG
601   AGAAACGGAT GCTGATATTA CTGTTGCTGC CTTGCCCATG
      GATGAGGAAC GTGCAACTGC ATTTGGCCTT ATGAAAATCG
      ATGAAGAAGG GAGGATAATT GAATTCGCAG AGAAACCAAA
      AGGAGAACAG TTGAAAGCTA TGATGGTTGA TACGACCATA
      CTTGGCCTTG AAGATGCGAG GGCAAAGGAA ATGCCTTATA
801   TTGCTAGCAT GGGTATCTAT GTTATTAGCA AACATGTGAT
      GCTTCAGCTT CTCCGTGAGC AATTTCCTGG AGCTAATGAC
      TTCGGAAGTG AAGTTATCCC TGGTGCAACT AGCACTGGCA
      TGAGGGTACA AGCATACCTA TACGACGGTT ACTGGGAAGA
      TATTGGTACA ATTGAGGCAT TCTATAATGC AAATTTGGGA
1001  ATTACCAAAA AACCAATACC TGATTTCAGT TTCTATGACC
      GTTCTGCTCC CATTTACACA CAACCTCGAC ACTTGCCTCC
      TTCAAAGGTT CTTGATGCTG ATGTGACAGA CAGTGTAATT
      GGTGAAGGAT GTGTTATTAA AAACTGCAAG ATACACCATT
      CAGTAGTTGG ACTCCGTTCC TGCATATCTG AAGGTGCAAT
1201  AATAGAGGAC ACGTTGCTAA TGGGTGCGGA CTACTATGAG
      ACTGAAGCTG ATAAGAAACT CCTTGCTGAA AAAGGTGGCA
      TTCCCATTGG TATTGGAAAG AATTCACACA TCAAAAGAGC
```

AATCATTGAC AAGAATGCTC GTATTGGAGA TAACGTGATG
ATAATCAATG TTGACAATGT TCAAGAAGCG GCGAGGGAGA
1401 CAGATGGATA CTTCATCAAA AGTGGCATCG TAACTGTGAT
CAAGGATGCT TTACTCCCTA GTGGAACAGT CATATGAAGC
AGATGTGAAA TGTATGCCAA AAGACAGGGC TACTTGCGTC
AGTCTGGAAT CAACCAACAA GGCCGCGAAG GAGATCATAA
AATAAAAA.G GAGTGCCATG CGAGTCACTT CTACACCCTT
1601 TTCCCCCCTT GATGTATTAG GAACTGTGAT GTACAAGCAA
CTGTGATGCA CTTACGCGAA GTGCCCCTGG ATTCAGCTTT
CTCTTTGCTT GTAACTGGTT TCCAGCAGAC CATGCTATTT
GTTGTATGGT TCGTGCAAAA CCTTGCGATG CTTTATATAT
GCTTTATATA TAAACAAGAT GAATCCCCGC GCGTTGCTGC
2001 GGCACAAAAA AAAAAAAAAA AA

In the event that this sequence contains an error, see the corresponding sequence in the accompanying figures.

(2c) INFORMATION FOR SEQUENCE I.D. 3

SEQUENCE TYPE:	ENZYME
MOLECULE TYPE:	AMINO ACID
ORIGINAL SOURCE:	BARLEY
SEQUENCE LENGTH:	528
TOPOLOGY:	LINEAR
SEQUENCE:	Derived amino acid sequence of a cDNA encoding the large subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (bepl10)

SEQ. ID. No. 3

1	M S S M Q F S S V L	P L E G K A C V S P
	V R R E G S A C E R	L K I G D S S S I R
	H E R A S R R M C N	G G A G A P P P P V
	R S A C S P P T P A	R P T P L F S G R P
	S G G I T P I R T R	S R P S V A A V I L
101	G G G T G T Q L F P	L T S T R A T P A V
	P I G G C Y R L I D	I P M S N C F N S G
	I N K I F V M T Q F	N S A S L N R H I H
	R T Y L G G G I N F	T D G S V E V L A A
	T Q M P G E A A G W	F R G T A D A V R K
201	F I W V L E D Y Y K	H K S I E H I L I L
	S G D Q L Y R M D Y	M E L V Q K H V D D
	N A D I T L S C A P	V G E S R A S E Y G
	L V K F D S S G R V	I Q F S E K P K G D
	D L E A M K V D T S	F L N F A I D D P A
301	K Y P Y I A S M G V	Y V F K R D V L L N
	L L K S R Y A E L H	D F G S E I L P R A
	L H D H N V Q A Y V	F T D Y W E D I G T
	I R S F F D A N M A	L C E Q P P K F E F
	Y D P K T P F F T S	P R Y L P P T K S D
401	K C R I K E A I I S	H G C F L R E C K I
	E H S I I G V R S R	L N S G S E L K N A
	M M M G A D S Y E T	E D E I S R L M S E
	G K V P I G V G E N	T K I S N C I I D M
	N A R I G R D V V I	S N K E G V Q E A D
501	R P E E G Y Y I R S	G I V V I Q K N A T
	I K D G T V V *	

In the event that this sequence contains an error, see the corresponding sequence in the accompanying figures.

(2d) INFORMATION FOR SEQUENCE I.D. 4

SEQUENCE TYPE:	ENZYME
MOLECULE TYPE:	AMINO ACID
ORIGINAL SOURCE:	BARLEY
SEQUENCE LENGTH:	472
TOPOLOGY:	LINEAR
SEQUENCE:	Derived amino acid sequence of a cDNA encoding the small subunit of ADP-glucose pyrophosphorylase from barley seed endosperm (beps)

SEQ. ID. No. 4

1	D V P L A S K V P L	P S P S K H E Q C N
	V Y S H K S S S K H	A D L N P H A I D S
	V L G I I L G G G A	G T R L Y P L T K K
	R A K P A V P L G A	N Y R L I D I P V S
	N C L N S N I S K I	Y V L T Q F N S A S
101	L N R H L S R A Y G	S N I G G Y K N E G
	F V E V L A A Q Q S	P D N P D W F Q G T
	A D A V R Q Y L W L	F E E H N V M E Y L
	I L A G D H L Y R M	D Y E K F I Q A H R
	E T D A D I T V A A	L P M D E E R A T A
201	F G L M K I D E E G	R I I E F A E K P K
	G E Q L K A M M V D	T T I L G L E D A R
	A K E M P Y I A S M	G I Y V I S K H V M
	L Q L L R E Q F P G	A N D F G S E V X P
	G A T S T G M R V Q	A Y L Y D G Y W E D
301	I G T I E A F Y N A	N L G I T K K P I P
	D F S F Y D R S A P	I Y T Q P R H L P P
	S K V L D A D V T D	S V I G E G C V I K
	N C K I H H S V V G	L R S C I S E G A I
	I E D T L L M G A D	Y Y E T E A D K K L
401	L A E K G G I P I G	I G K N S H I K R A
	I I D K N A R I G D	N V M I I N V D N V
	Q E A A R E T D G Y	F I K S G I V T V I
	K D A L L P S G T V	I *

In the event that this sequence contains an error, see the corresponding sequence in the accompanying figures.

(2e) INFORMATION FOR SEQUENCE I.D. 5

SEQUENCE TYPE:	NUCLEIC ACID
MOLECULE TYPE:	DNA
ORIGINAL SOURCE:	POTATO
SEQUENCE LENGTH:	480
STRANDEDNESS:	DOUBLE
TOPOLOGY:	LINEAR
SEQUENCE:	Starch branching enzyme cDNA (First 480 nucleotides from 5' end)

SEQ. ID. No. 5

```
1     CCCGTCTGTA  AGCATCATTA  GTGATGTTGT
31    TCCAGCTGAA  TGGGATGATT  CAGATGCAAA
61    CGTCTGGGGT  GAGAACATAC  AAGAAGGCAG
91    CAGCTGAAGC  AAAGTACCAT  AATTTAATCA
121   ATGGAAATTA  ATTTCAAAGT  TTTATCAAAA
151   CCCATTCGAG  GATCTTTTCC  ATCTTTCTCA
      CCTAAAGTTT  CTTCAGGGGC  TTCTAGAAAT
      AAGATATGTT  TTCCTTCTCA  ACATAGTACT
      GGACTGAAGT  TTGGATCTCA  GGAACGGTCT
      TGGGATATTT  CTTCCACCCC  AAAATCAAGA
301   GTTAGAAAAG  ATGAAAGGAT  GAAGCACAGT
      TCAGCTATTT  CCGCTGTTTT  GACCGATGAC
      AATTCGACAA  TGGCACCCCT  AGAGGAAGAT
      GTCAAGACTG  AAAATATTGG  CCTCCTAAAT
      TTGGATCCAA  CTTTGGAACC  TTATCTAGAT
451   CACTTCAGAC  ACAGAATGAA  GAGATATGTG
```

In the event that this sequence contains an error, see the corresponding sequence in the accompanying figures.

(2f) INFORMATION FOR SEQUENCE I.D. 6

SEQUENCE TYPE:	PEPTIDE
MOLECULE TYPE:	AMINO ACID
ORIGINAL SOURCE:	POTATO
SEQUENCE LENGTH:	120
TOPOLOGY:	LINEAR
SEQUENCE:	Starch branching enzyme amino acid (First 120 amino acids from amino terminus)

SEQ. ID. No. 6

1	MET Glu Ile Asn Phe Lys Val Leu Ser Lys
11	Pro Ile Arg Gly Ser Phe Pro Ser Phe Ser
21	Pro Lys Val Ser Ser Gly Ala Ser Arg Asn
31	Lys Ile Cys Phe Pro Ser Gln His Ser Thr
41	Gly Leu Lys Phe Gly Ser Gln Glu Arg Ser
51	Trp Asp Ile Ser Ser Thr Pro Lys Ser Arg
61	Val Arg Lys Asp Glu Arg MET Lys His Ser
71	Ser Ala Ile S er Ala Val Leu Thr Asp Asp
81	Asn Ser Thr MET Ala Pro Leu Glu Glu Asp
91	Val Lys Thr Glu Asn Ile Gly Leu Leu Asn
101	Leu Asp Pro Thr Leu Glu Pro Tyr Leu Asp
111	His Phe Arg His Arg MET Lys Arg Tyr Val

In the event that this sequence contains an error, see the corresponding sequence in the accompanying figures.

(2g) INFORMATION FOR SEQUENCE I.D. 7

SEQUENCE TYPE:	NUCLEIC ACID
MOLECULE TYPE:	DNA
ORIGINAL SOURCE:	POTATO
SEQUENCE LENGTH:	1047
STRANDEDNESS:	DOUBLE
TOPOLOGY:	LINEAR
SEQUENCE:	Tuber specific class 1 promoter

SEQ. ID. No. 7

```
1     TTGTTAGTTA ATGCGTATTA GTTTTAGCGA CGAAGCACTA
      AATCGTCTTT GTATACTTTC AGTGACACAT GTTTAGTGAC
      GACTGATTGA CGAAATTTTT TTCGTCTCAC AAAATTTTTA
      GTGACGAAAC ATGATTTATA GATGACGAAA TTATTTGTCC
      CTCATAATCT AATTTGTTGT AGTGATCATT ACTCCTTTGT
201   TTGTTTTATT TGTCATGTTA GTTCATTAAA AAAAAAATCT
      CTCTTCTTAT CAATTCTGAC GTGTTTAATA TCATAAGATT
      AAAAAATATT TTAATATATC TTTAATTTAA AGCCACAAAA
      TTTAAATTTC TTCGTTAACA TAATTTGTCA AATCAGGCTC
      AAAGATCGTT TTTCATATCG GAATCAGGAT TTTATTTATT
401   CTTTTAAAAA TAAAGAGGTG GTGAGCTAAA CAATTTCAAA
      TCTCATCACA CATATGGGGT CAGCCACAAA AATAAAGAAC
      GGTTGGAACG GATCTATTAT ATAATACTAA TAAAGAATAG
      AAAAAGGAAA GTGAGTGAGG TGCGAGGGAG AGAATCTGTT
      TAATATGCAG AGTCGATCAT GTGTCAGTTT TATCGATATG
601   ACTCTGATTT CAACTGAGTT TAAGCAATTC TGATAAGGCG
      AGGAAAATCA CAGTGCTGAA ATCTAGAAAA ATCTCATACA
      GTGAGATAAA TCTCAACAAA AACGTTGAGT CCATAGAGGG
      GGTGTATGTG ACACCCAACC TCAGCAAAAG AAAACCTCCC
      CTCAAGAAGG ACATTTGCGG TGCTAAACAA TTTCAAGTCT
801   CATCACACAT ATATATTATA TAATACTAAT AAAGAATAGA
      AAAAGGAAAG GTAAACATCA CTAATGACAG TTGCGGTGCA
      AAGTGAGTGA GATAATAAAC ATCAGTAATA GACATCACTA
      ACTTTTATTG GTTATGTCTT TCTCAAAATA AAATTTCTCA
      ACTTGTTTAC GTGCCTATAT ATACCATGCT TGTTATATGC
1001  TCAAAGCACC AACAAAATTT AAAAACACTT TGAACATTTG
      CCCCGGG
```

In the event that this sequence contains an error, see the corresponding sequence in th accompanying figures.

(2h) INFORMATION FOR SEQUENCE I.D. 8

SEQUENCE TYPE:	ENZYME CONSTRUCT
MOLECULE TYPE:	AMINO ACID
ORIGINAL SOURCE:	SPINACH and BARLEY
SEQUENCE LENGTH:	120
TOPOLOGY:	LINEAR
SEQUENCE:	Amino terminal amino acid sequence of the rubisco activase - AGP small subunit fusion enzyme.

SEQ.ID.NO.8

1	MATAVSTVGA	ATRAPLNLNG	SSAGASVPTS	GFLGSSLKKH	40
41	TNVRFPSSSR	TTSMTVKAAE	NEEKNTDKWA	HLAKDFSDDQ	80
81	LDIRRGKGMV	DSLGSMDVPL	ASKVPLPSPS	KHEQCNVYSH	120

(2i) INFORMATION FOR SEQUENCE I.D. 9

SEQUENCE TYPE:	ENZYME CONSTRUCT
MOLECULE TYPE:	AMINO ACID
ORIGINAL SOURCE:	SPINACH and BARLEY
SEQUENCE LENGTH:	120
TOPOLOGY:	LINEAR
SEQUENCE:	**Amino terminal amino acid sequence of the rubisco activase - AGP large subunit fusion enzyme.**

SEQ.ID.NO.9

1	MATAVSTVGA ATRAPLNLNG SSAGASVPTS GFLGSSLKKH	40
41	TNVRFPSSSR TTSMTVKAAE NEEKNTDKWA HLAKDFSDDQ	80
81	LDIRRGKGMV DSLGIHMQFS SVLPLEGKAC VSPVRREGSA	120

(2j) INFORMATION FOR SEQUENCE I.D. 10

SEQUENCE TYPE:	ENZYME CONSTRUCT
MOLECULE TYPE:	AMINO ACID
ORIGINAL SOURCE:	SPINACH and BARLEY
SEQUENCE LENGTH:	120
TOPOLOGY:	LINEAR
SEQUENCE:	Amino terminal amino acid sequence of the starch branching enzyme - AGP large subunit fusion enzyme.

SEQ.ID.NO.10

1	MEINFKVLSK PIRGSFPSFS PKVSSGASRN KICFPSQHST	40
41	GLKFGSQERS WDISSTPKSR VRKDERMKHS SAISAVLTDD	80
81	NSTMAPLEED VKTENIGLLN LDPMQFSSVL PLEGKACVSP	120

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 6.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23. St. Machar Drive
Aberdeen
Scotland
United Kingdom
AB2 1RY

Date of deposit	Accession Number
29 March 1994	NCIMB 40618

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 8.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23. St. Machar Drive
Aberdeen
Scotland
United Kingdom
AB2 1RY

Date of deposit	Accession Number
29 March 1994	NCIMB 40619

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet [ ]

In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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A. The indications made below relate to the microorganism referred to in the description on page 19, line 10.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution (*including postal code and country*)

23. St. Machar Drive
Aberdeen
Scotland
United Kingdom
AB2 1RY

Date of deposit	Accession Number
29 March 1994	NCIMB 40620

C. ADDITIONAL INDICATIONS (*leave blank if not applicable*) — This information is continued on an additional sheet [ ]

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

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A. The indications made below relate to the microorganism referred to in the description on page 19, line 12.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23. St. Machar Drive
Aberdeen
Scotland
United Kingdom
AB2 1RY

Date of deposit	Accession Number
29 March 1994	NCIMB 40621

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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A. The indications made below relate to the microorganism referred to in the description on page 19, line 14.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23. St. Machar Drive
Aberdeen
Scotland
United Kingdom
AB2 1RY

Date of deposit	Accession Number
29 March 1994	NCIMB 40622

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 21.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23. St. Machar Drive
Aberdeen
Scotland
United Kingdom
AB2 1RY

Date of deposit	Accession Number
31 March 1994	NCIMB 40623

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, line 25.

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet [X]

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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Form PCT/RO/134 (July 1992)

The claims defining the invention are as follows:

1. A transgenic starch producing plant or alga comprising a nucleotide sequence coding for an exogenous ADP glucose pyrophosphorylase (AGP) enzyme or a sub-unit thereof which retains the enzymatic activity of the AGP enzyme supplemented with a plastid transit peptide, wherein the nucleotide sequence is capable of being expressed in the plant or alga; and wherein the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and any *in vivo* level of inorganic phosphate; and wherein the activity of the enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

2. A plant or alga according to claim 1 wherein the AGP enzyme is a heteromer, preferably a heterotetramer, more preferably a heteromer containing two large sub-units and two small sub-units.

3. A plant or alga according to claim 1 or claim 2 wherein the AGP enzyme is isolatable from <u>Hordeum</u>, preferably wherein the enzyme is barley <u>(Hordeum vulgare)</u> endosperm AGP or a sub-unit thereof, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units.

4. A plant or alga according to any one of claims 1 to 3 wherein the nucleotide sequence is a DNA sequence.

5. A plant or alga according to claim 4 wherein the DNA sequence encodes the large sub-unit of the barley endosperm AGP or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequence defining the large sub-unit of the barley endosperm AGP.

6. A plant or alga according to claim 4 or claim 5 wherein the DNA sequence is the sequence identified herein as SEQ ID No. 1, including non-critical allelic variations of that sequence.

7. A plant or alga according to claim 4 wherein the DNA sequence encodes the small sub-unit of the barley endosperm AGP, or a variant th reof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequence defining the small sub-unit of the barley endosperm AGP.

8. A plant or alga according to claim 4 or claim 7 wherein th DNA sequence

is the sequence identified herein as SEQ ID NO: 2, including non-critical allelic variations of that sequence.

9. A plant or alga according to claim 4 comprising a DNA sequence according to claim 5 or claim 6 and a DNA sequence according to claim 7 or claim 8, preferably wherein each DNA sequence additionally codes for a different marker.

10. A plant or alga according to any one of claims 1 to 9 wherein the expressed AGP enzyme or sub-unit thereof comprises the amino acid sequence set out in SEQ ID NO: 3, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

11. A plant or alga according to any one of claims 1 to 9 wherein the expressed AGP enzyme or sub-unit thereof comprises the amino acid sequence set out in SEQ ID NO: 4, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

12. A plant or alga according to claim 1 wherein the expressed AGP comprises both a large sub-unit having the amino acid sequence set out in SEQ ID NO: 3 or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence, and a small sub-unit having the amino acid sequence set out in SEQ ID NO: 4 or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

13. A plant or alga according to any one of claims 1 to 12 wherein the transit peptide is Rubisco Activase transit peptide or Starch Branching enzyme transit peptide.

14. A plant or alga according to claim 13 wherein the transit peptide is coded for by a DNA sequence comprising the sequence identified as SEQ ID No. 5, including non-critical allelic variations of that sequence.

15. A plant or alga according to claim 13 wherein the transit peptide has an amino acid sequence comprising the sequence identified as SEQ ID NO: 6, or a variant thereof having non-critical amino acid substitution(s) or del-tion(s) at one or mor locations in that sequence.

16. A plant or alga according to any on of claims 1 to 15 wherein the nucleotide sequence is operatively connected to a promoter which express s th

nucleotide sequence wherein the promoter is cell, tissu or organ specific.

17. A plant or alga according to claim 16 wh rein the promoter has the sequence identified as SEQ ID NO: 7 or a variant thereof having non-critical nucleotide substitution(s) or deletion(s) at one or more locations in that sequence.

18. A plant or alga according to claim 1 wherein the AGP enzyme or sub-unit thereof comprises the amino acid sequence set out in SEQ ID NO: 3, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence and/or the amino acid sequence set out in SEQ ID NO: 4, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more locations in that sequence.

19. A plant or alga according to any one of claims 1 to 18 wherein the plant or alga is a transgenic plant.

20. A plant or alga according to claim 19 wherein the transgenic plant is a potato plant.

21. A potato tuber containing an enhanced starch content, when obtained from a transgenic potato plant according to claim 20.

22. A method of increasing the rate and/or yield of starch production in a plant or alga, especially a plant or a plant cell, which method comprises introducing into an organism a nucleotide sequence as defined in claim 1 or any claim dependent thereon to form a transgenic organism as defined in any one of the preceding claims and expressing the nucleotide sequence.

23. An isolated barley (Hordeum vulgare) AGP enzyme or sub-unit thereof supplemented with a plasmid transit peptide, whose *in vivo* activity is substantially independent of any level of an *in vivo* 3-phosphoglycerate and/or any *in vivo* lev l of inorganic phosphate, and whose activity is not stimulated by fructose-1,6-biphosphate and/or is not inhibited by AMP.

24. Plasmid pPPS4.

25. Plasmid pPPL4.

26. Plasmid pPPL5.

27. A method of increasing the rate and/or yield of starch production in a plant or alga, especially a plant or a plant cell, which method comprises introducing into a plant or alga a recombinant DNA construct containing an exog nous DNA

sequence encoding an exog nous ADP glucose pyrophosphorylase nzyme (AGP) or sub-unit thereof and one or more promoter sequ nces enabling the expression of the AGP encoded by that sequence by the organism thereby to increase the AGP content of the organism and in consequence to increase the rate of starch production by the organism and/or the starch yield, wherein the said DNA sequence is the gene sequence encoding the barley (Hordeum vulgare) endosperm AGP or a sub-unit thereof supplemented with a plasmid transit peptide or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units, wherein the construct is expressed in the organism; wherein the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and/or any *in vivo* level of inorganic phosphate; and further wherein the activity of the enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and/or is not inhibited by AMP.

28. A vector for the transformation of a plant or alga, especially a plant or a plant cell, to increase the AGP content of such a plant or alga consequently to increase the rate of starch production by such a plant or alga, such vector comprising a recombinant DNA construct containing a DNA sequence encoding an exogenous ADP glucose pyrophosphorylase enzyme (AGP), such vector also incorporating the necessary promoter and other sequences enabling the expression of that exogenous AGP in a plant or alga transformed by that vector, wherein the said DNA sequence is the gene sequence encoding the barley (Hordeum vulgare) endosperm AGP or a sub-unit thereof supplemented with a plasmid transit peptide, or a variant thereof having non-critical amino acid substitution(s) or deletion(s) at one or more points in the amino acid sequences defining the barley endosperm AGP or either of its sub-units, wherein the construct is capable of being expressed in the organism; wherein the activity of the enzyme or sub-unit thereof is substantially independent of any level of *in vivo* 3-phospho-glycerate and any *in vivo* level of inorganic phosphate; and further wherein th activity of th enzyme or sub-unit thereof is not stimulated by fructose-1,6-bisP and is not inhibited by AMP.

29. A foodstuff made from or comprising a plant or alga according to claim 1 or any claim dependent thereon.

30. A foodstuff according to claim 29 wherein the foodstuff is a fried foodstuff.

31. A foodstuff according to claim 29 or claim 30 wherein the foodstuff is a potato.

32. A plant or alga according to claim 1 substantially as hereinbefore described with reference to any one of the examples.

Dated: 15 May, 1998

DANISCO A/S
By their Patent Attorneys
PHILLIPS ORMONDE & FITZPATRICK

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65392/94

FIG. 1

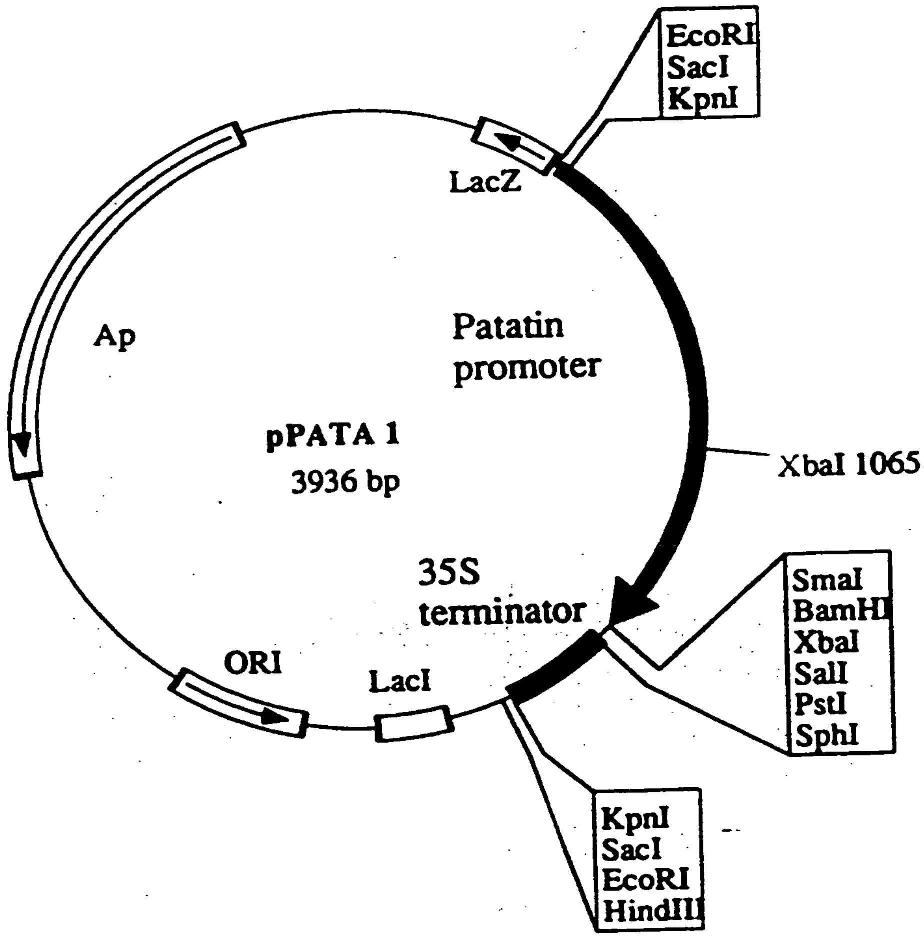
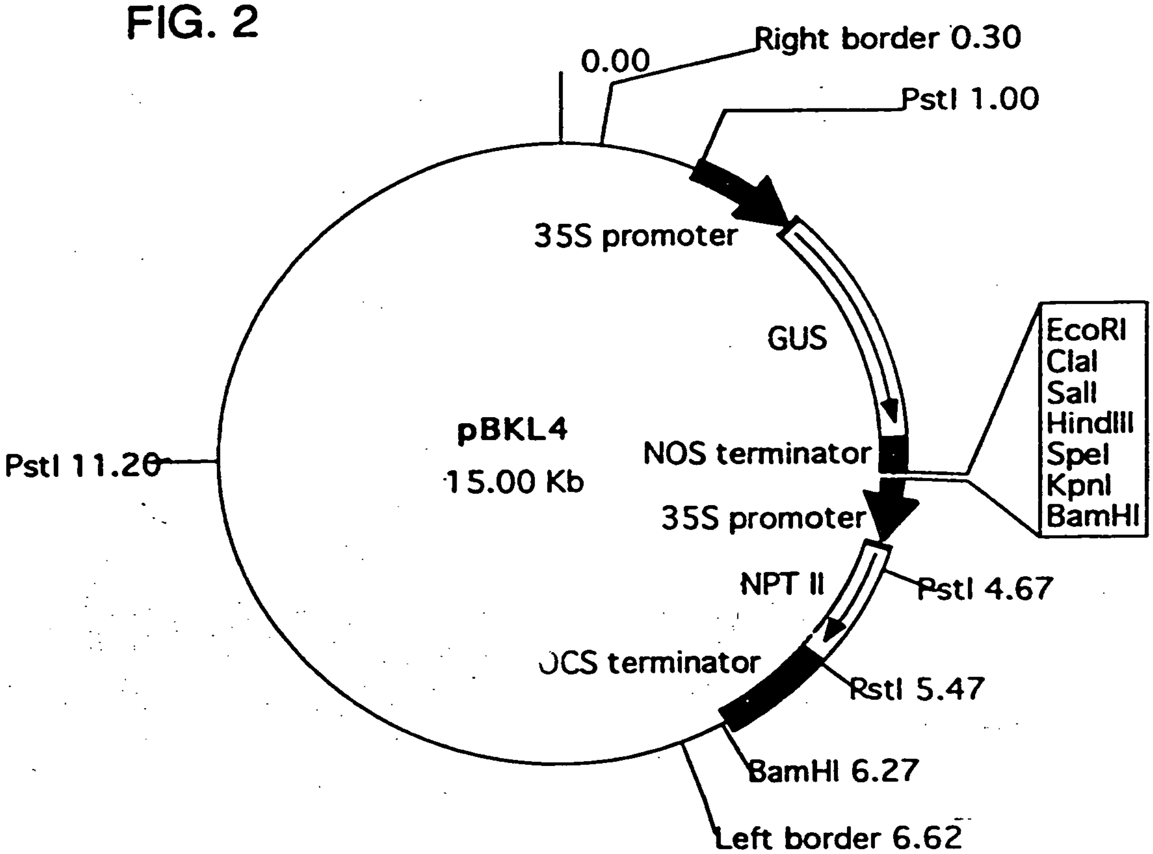


FIG. 2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SalI
HindIII
SpeI
KpnI
BamHI
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OCS terminator
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BamHI 6.27
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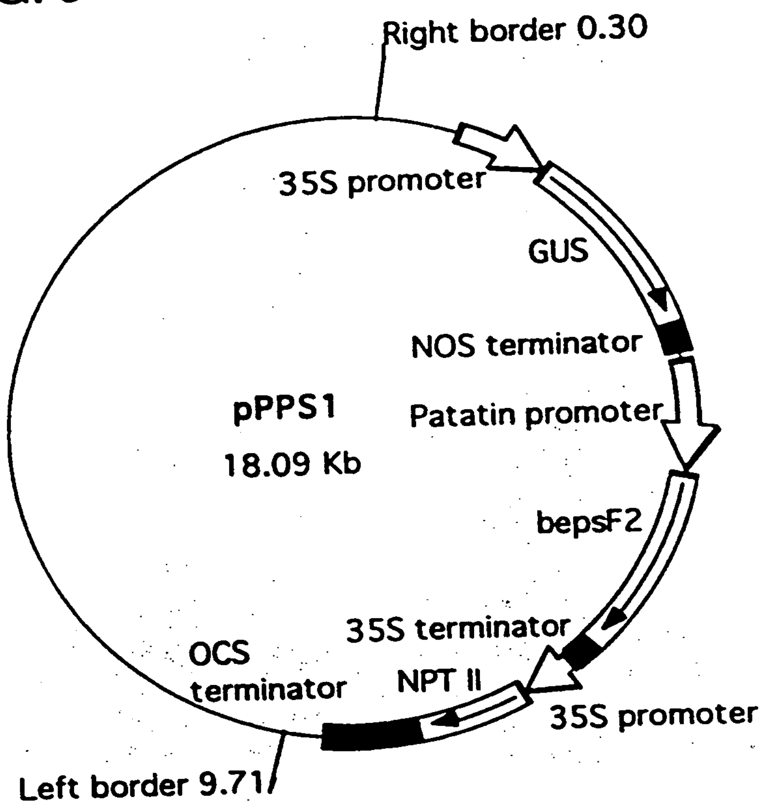
FIG. 3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terminator
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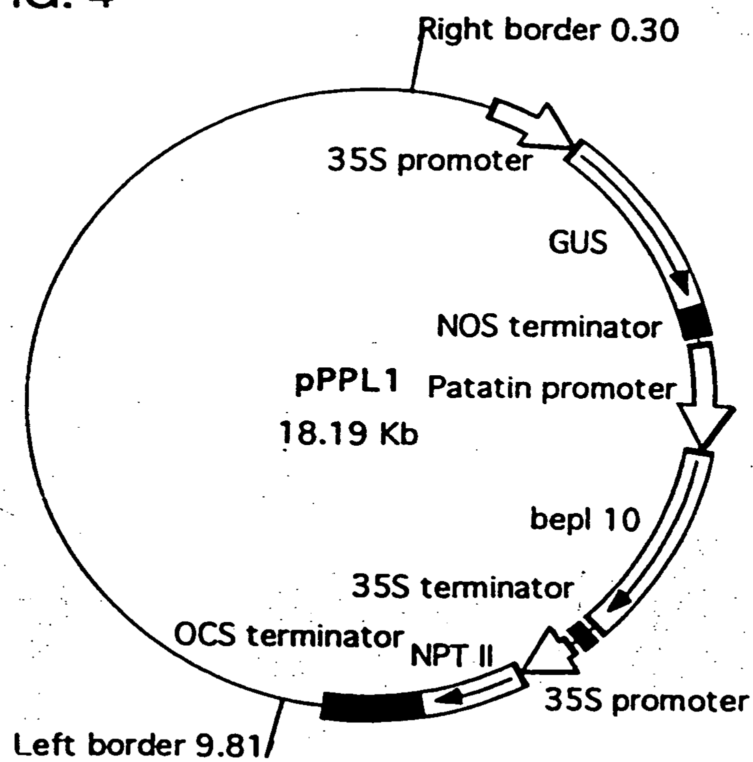
FIG. 4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NOS terminator
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Patatin promoter
bepl 10
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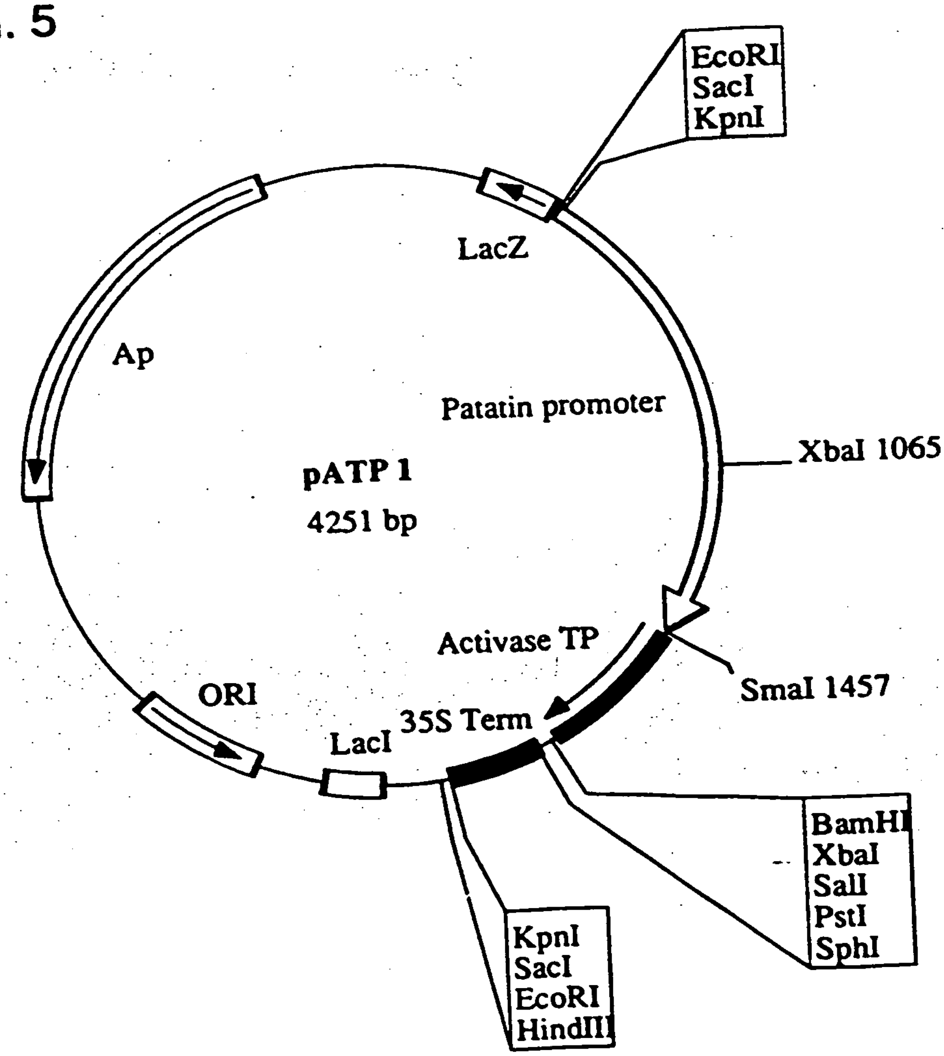
Left border 9.81

FIG. 5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Ap
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SmaI 1457
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XbaI
SalI
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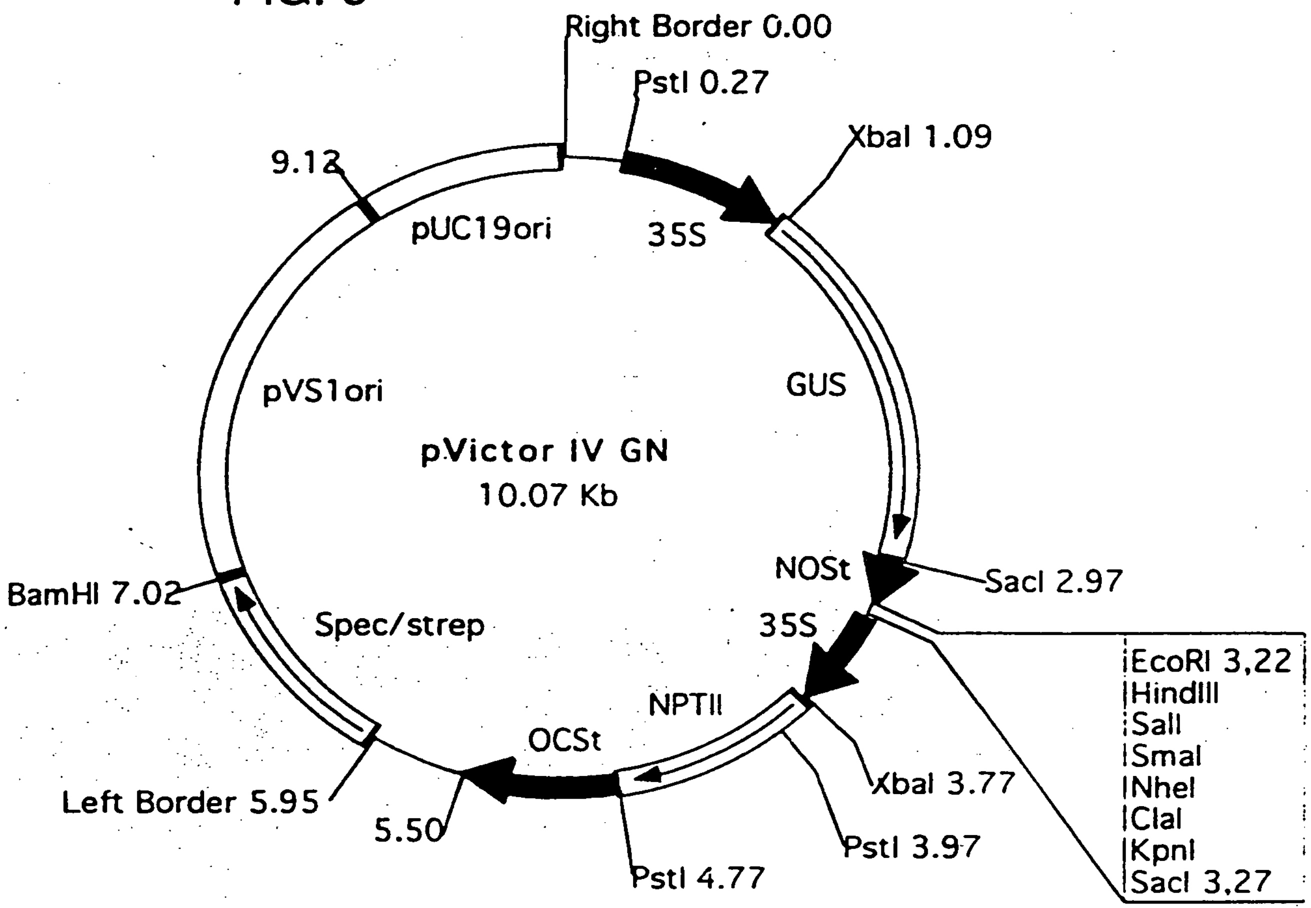
KpnI
SacI
EcoRI
HindIII

FIG. 6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XbaI 1.09
9.12
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35S
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pVS1ori
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NOSt
SacI 2.97
BamHI 7.02
Spec/strep
35S
EcoRI 3,22
HindIII
SalI
SmaI
NheI
ClaI
KpnI
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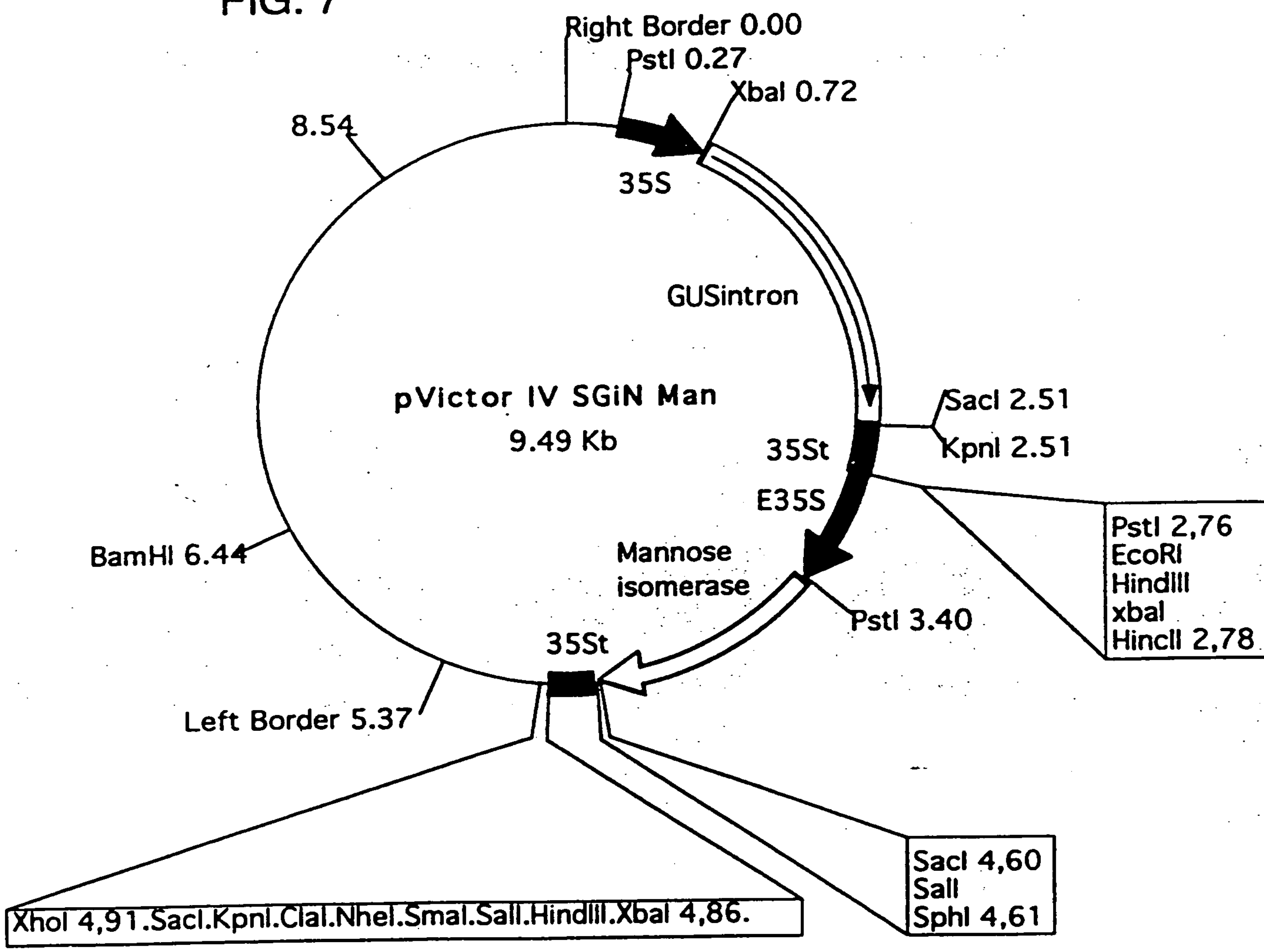
PstI 4.77

FIG. 7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pVictor IV SGiN Man
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KpnI 2.51
35St
E35S
PstI 2,76
EcoRI
HindIII
xbaI
HincII 2,78
Mannose
isomerase
PstI 3.40
BamHI 6.44
35St
Left Border 5.37
SacI 4,60
SalI
SphI 4,61
XhoI 4,91.SacI.KpnI.ClaI.NheI.SmaI.SalI.HindIII.XbaI 4,86.

FIG 8

8.1 Amino terminal amino acid sequence of the rubisco activase - AGP small subunit fusion enzyme

1 MATAVSTVGA ATRAPLNLNG SSAGASVPTS GFLGSSLKKH 40
41 TNVRFPSSSR TTSMTVKAAE NEEKNTDKWA HLAKDFSDDQ 80
81 LDIRRGKGMV DSLGSMDVPL ASKVPLPSPS KHEQCNVYSH 120

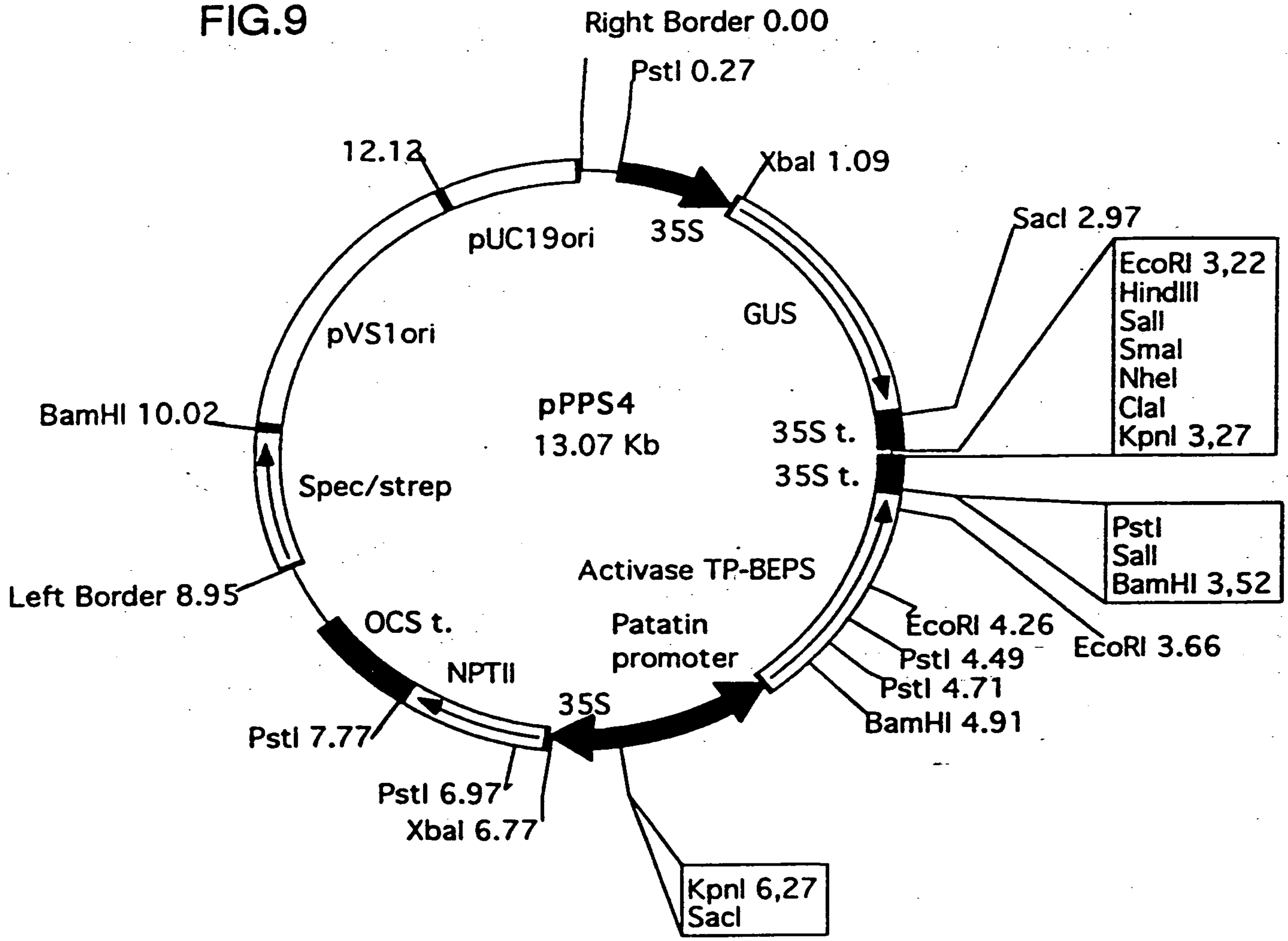
8.2 Amino terminal amino acid sequence of the rubisco activase - AGP large subunit fusion enzyme

1 MATAVSTVGA ATRAPLNLNG SSAGASVPTS GFLGSSLKKH 40
41 TNVRFPSSSR TTSMTVKAAE NEEKNTDKWA HLAKDFSDDQ 80
81 LDIRRGKGMV DSLGIHMQFS SVLPLEGKAC VSPVRREGSA 120

8.3 Amino terminal amino acid sequence of the starch branching enzyme - AGP large subunit fusion enzyme

1 MEINFKVLSK PIRGSFPSFS PKVSSGASRN KICFPSQHST 40
41 GLKFGSQERS WDISSTPKSR VRKDERMKHS SAISAVLTDD 80
81 NSTMAPLEED VKTENIGLLN LDPMQFSSVL PLEGKACVSP 120

FIG.9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SalI
SmaI
NheI
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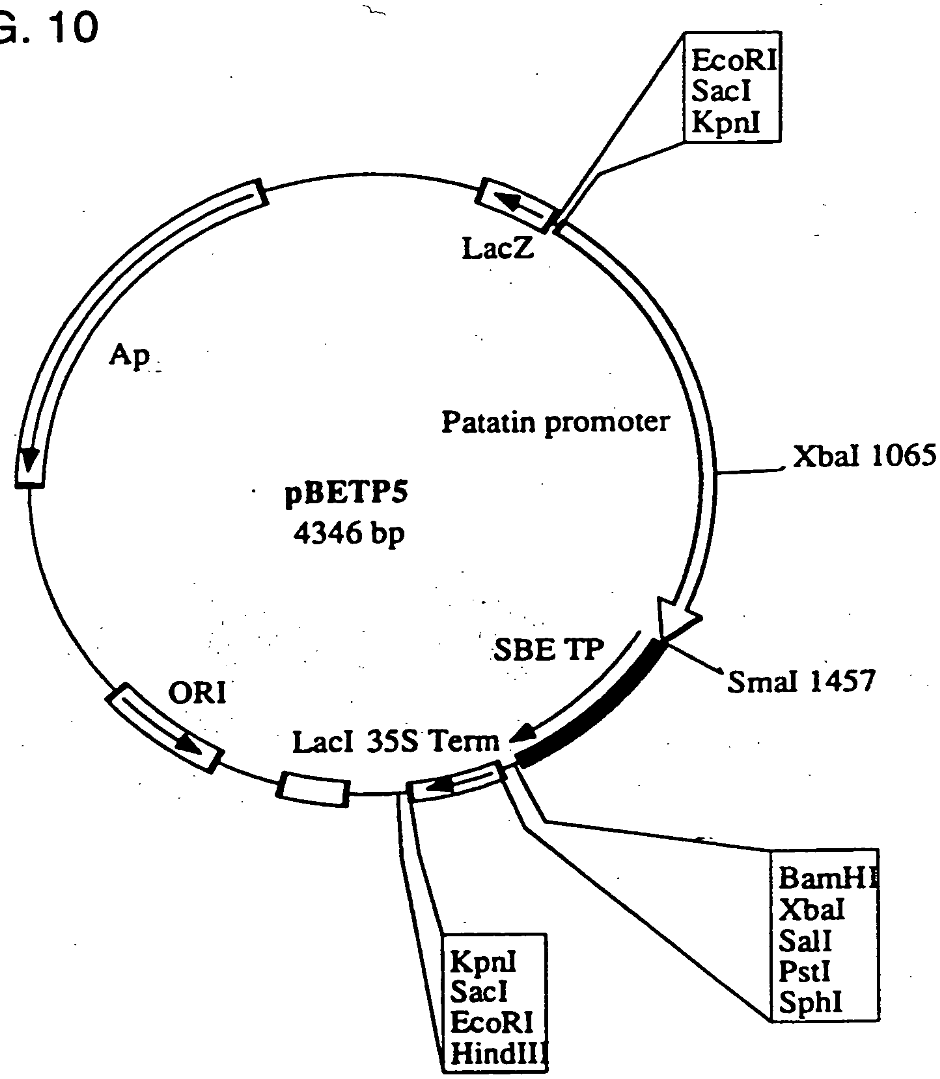
PstI
SalI
BamHI 3,52
EcoRI 3.66
EcoRI 4.26
PstI 4.49
PstI 4.71
BamHI 4.91
KpnI 6,27
SacI
XbaI 6.77
PstI 6.97
PstI 7.77
Left Border 8.95
BamHI 10.02
12.12
pUC19ori
35S
GUS
pVS1ori
pPPS4
13.07 Kb
35S t.
35S t.
Spec/strep
Activase TP-BEPS
Patatin
promoter
OCS t.
NPTII
35S

FiG. 10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LacZ
Ap
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pBETP5
4346 bp
SBE TP
SmaI 1457
ORI
LacI 35S Term
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XbaI
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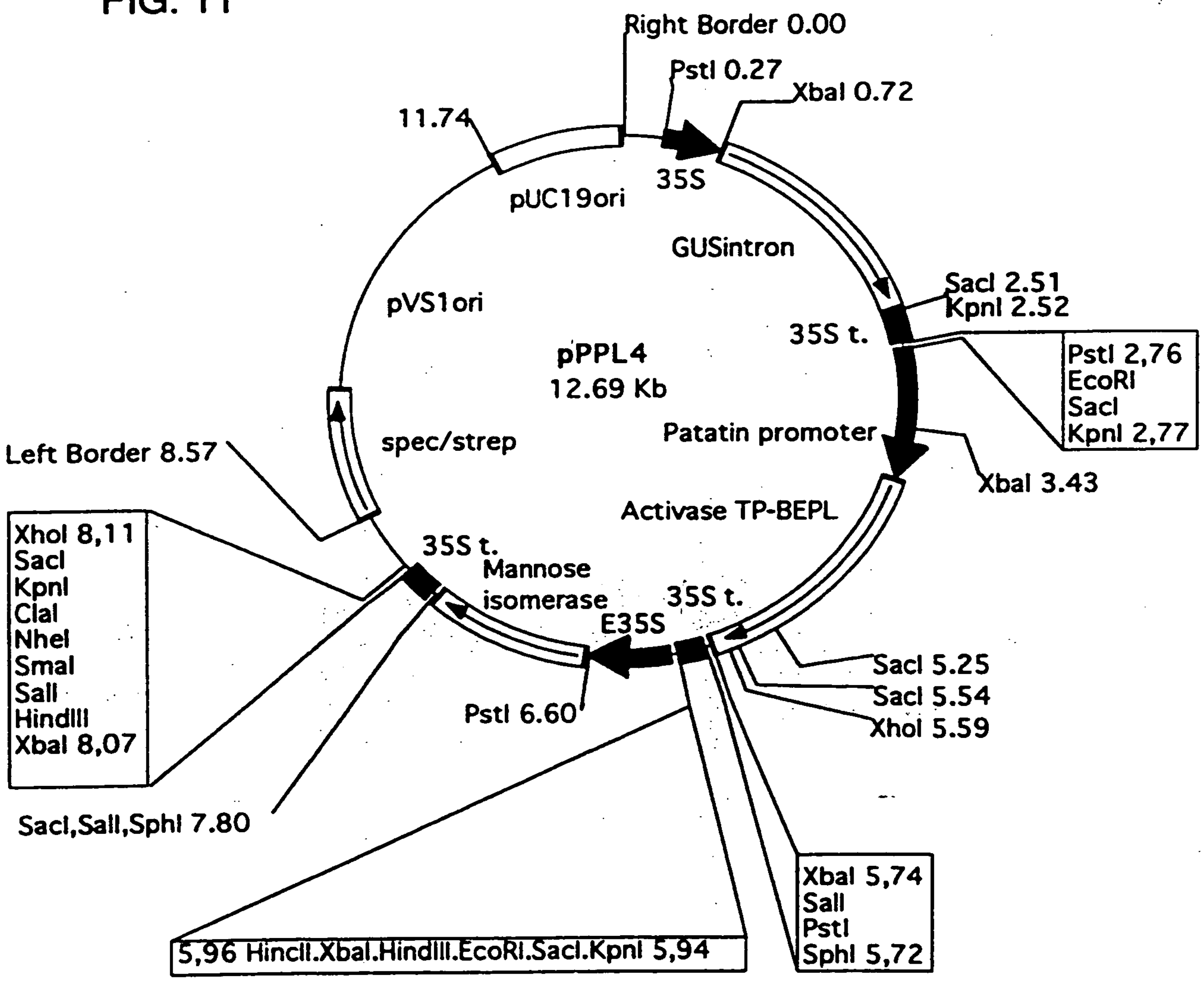
PstI
SphI
KpnI
SacI
EcoRI
HindIII

FIG. 11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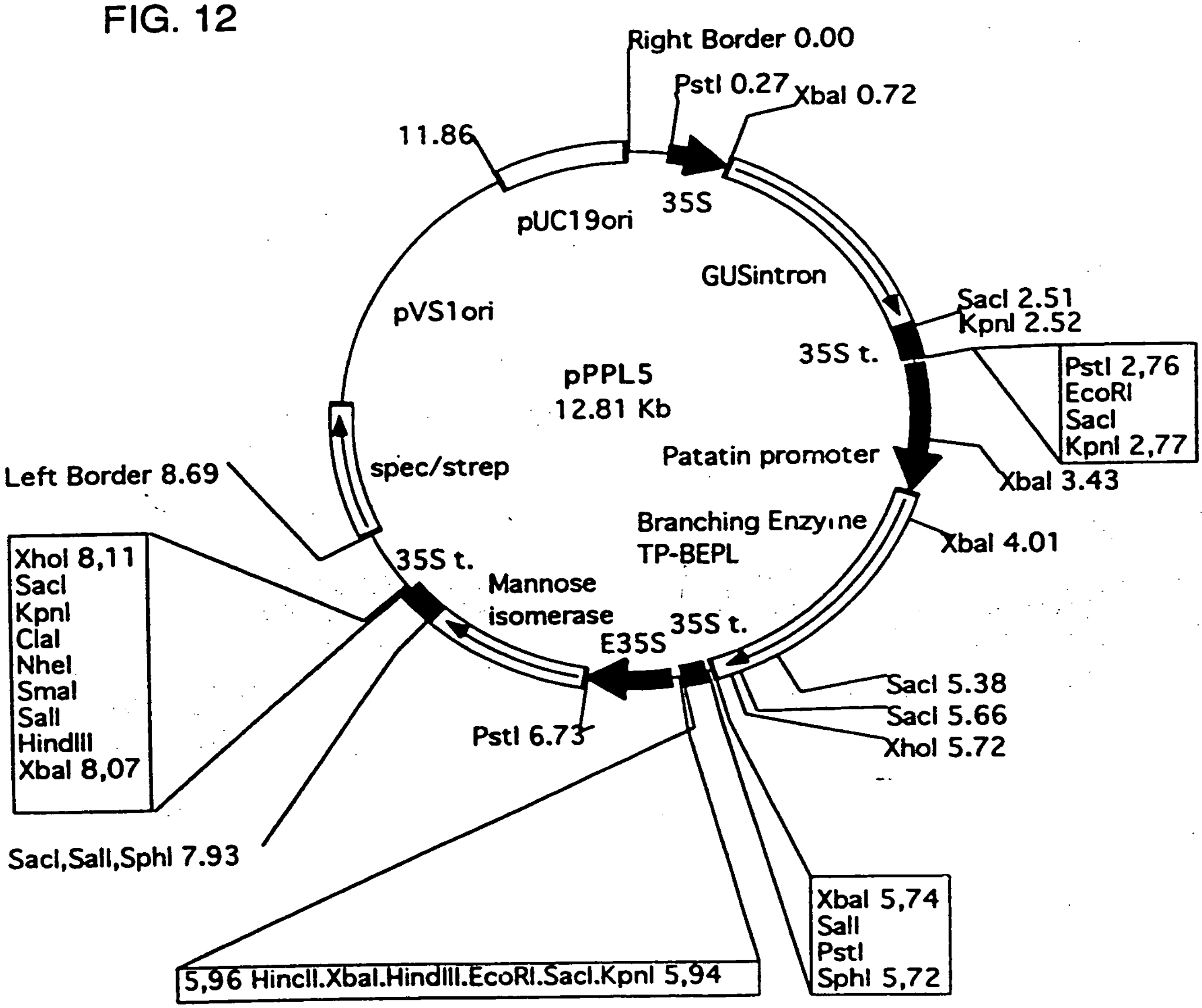
pPPL4
12.69 Kb
PstI 2,76
EcoRI
SacI
KpnI 2,77
spec/strep
Patatin promoter
Left Border 8.57
XbaI 3.43
Activase TP-BEPL
XhoI 8,11
SacI
KpnI
ClaI
NheI
SmaI
SalI
HindIII
XbaI 8,07
35S t.
Mannose
isomerase
35S t.
E35S
SacI 5.25
SacI 5.54
XhoI 5.59
PstI 6.60
SacI,SalI,SphI 7.80
XbaI 5,74
SalI
PstI
SphI 5,72
5,96 HincII.XbaI.HindIII.EcoRI.SacI.KpnI 5,94

FIG. 12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GUSintron
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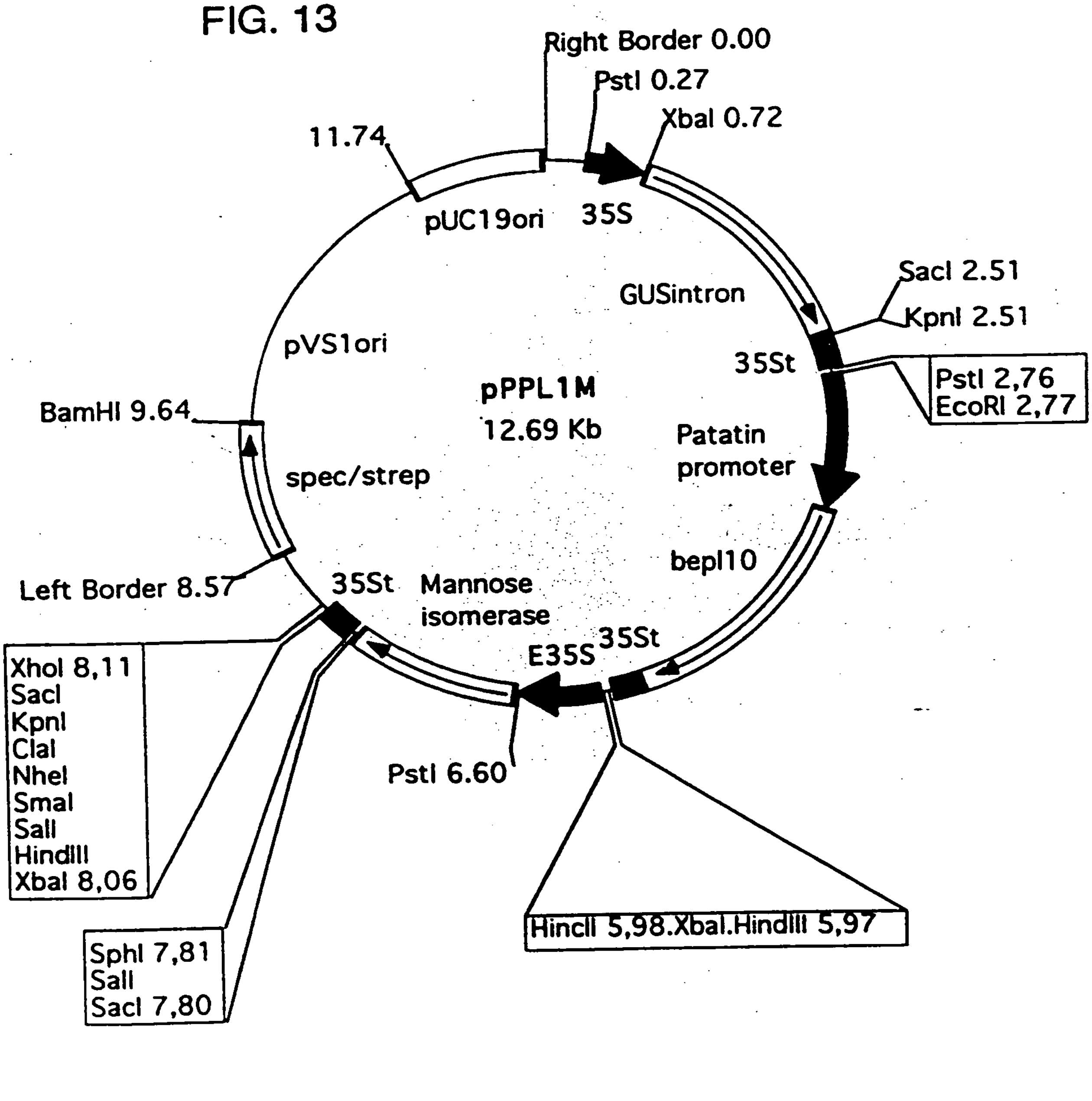
KpnI 2.52
pVS1ori
35S t.
PstI 2,76
EcoRI
SacI
KpnI 2,77
pPPL5
12.81 Kb
Patatin promoter
XbaI 3.43
Left Border 8.69
spec/strep
Branching Enzyme
TP-BEPL
XbaI 4.01
XhoI 8,11
SacI
KpnI
ClaI
NheI
SmaI
SalI
HindIII
XbaI 8,07
35S t.
Mannose
isomerase
E35S
35S t.
SacI 5.38
SacI 5.66
XhoI 5.72
PstI 6.73
SacI,SalI,SphI 7.93
XbaI 5,74
SalI
PstI
SphI 5,72
5,96 HincII.XbaI.HindIII.EcoRI.SacI.KpnI 5,94

FIG. 13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GUSintron
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KpnI 2.51
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PstI 2,76
EcoRI 2,77
pVS1ori
pPPL1M
12.69 Kb
Patatin
promoter
BamHI 9.64
spec/strep
bepl10
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35St
Mannose
isomerase
35St
E35S
XhoI 8,11
SacI
KpnI
ClaI
NheI
SmaI
SalI
HindIII
XbaI 8,06
PstI 6.60
HincII 5,98.XbaI.HindIII 5,97
SphI 7,81
SalI
SacI 7,80

FIG. 14

Nucleotide sequence and derived amino acid sequence of a cDNA encoding the large subunit of ADP-glucose pyrophosphorylase from barley seed endosperm.

```
        10                  30                  50
         .         .         .         .         .         .
ACGACCACCTCCGAACTCAACGCCTCCACGGACCATCTCTCTCCTCTCCCCTCCCCTCAC

        70                  90                 110
         .         .         .         .         .         .
CACCACCACCACCACCACCCCTTCTCCCTCCCTGCATTTGATTCGTTCATATTCATCCGT

       130                 150                 170
         .         .         .         .         .         .
CGCTTGCCCCGGTCGCCACCCCGTCGATCCCTCACCCCGCCGTCCCGGCAGTTGCAGGTG

       190                 210                 230
         .         .         .         .         .         .
GACTGCTAATGTCATCGATGCAGTTCAGCAGCGTGCTGCCCCTGGAGGGCAAGGCGTGCG
          M  S  S  M  Q  F  S  S  V  L  P  L  E  G  K  A  C  V

       250                 270                 290
         .         .         .         .         .         .
TTTCCCCAGTCAGGAGAGAGGGATCGGCCTGCGAGCGCCTCAAGATCGGGGACAGCAGCA
  S  P  V  R  R  E  G  S  A  C  E  R  L  K  I  G  D  S  S  S

       310                 330                 350
         .         .         .         .         .         .
GCATCAGGCACGAGAGAGCGTCCAGGAGGATGTGCAACGGCGGCGCAGGGGCCCCGCCGC
  I  R  H  E  R  A  S  R  R  M  C  N  G  G  A  G  A  P  P  P

       370                 390                 410
         .         .         .         .         .         .
CACCGGTGCGCAGTGCGTGCTCACCTCCGACGCCAGCCCGGCCGACACCCTTGTTCTCCG
  P  V  R  S  A  C  S  P  P  T  P  A  R  P  T  P  L  F  S  G

       430                 450                 470
         .         .         .         .         .         .
GACGTCCTTCCGGAGGAATTACGCCGATCCGAACGAGGTCGCGGCCGTCGGTCGCGGCCG
  R  P  S  G  G  I  T  P  I  R  T  R  S  R  P  S  V  A  A  V

       490                 510                 530
         .         .         .         .         .         .
TCATACTCGGCGGCGGCACCGGGACTCAGCTCTTCCCGCTCACAAGCACAAGGGCCACAC
  I  L  G  G  G  T  G  T  Q  L  F  P  L  T  S  T  R  A  T  P
```

```
         550                 570                 590
           .         .         .         .         .         .
CTGCTGTTCCTATTGGAGGATGTTACAGGCTCATCGATATTCCCATGAGCAACTGCTTCA
  A  V  P  I  G  G  C  Y  R  L  I  D  I  P  M  S  N  C  F  N

         610                 630                 650
           .         .         .         .         .         .
ACAGTGGCATCAACAAGATATTCGTCATGACCCAGTTCAACTCGGCATCTCTCAATCGCC
  S  G  I  N  K  I  F  V  M  T  Q  F  N  S  A  S  L  N  R  H

         670                 690                 710
           .         .         .         .         .         .
ACATTCACCGCACCTACCTCGGCGGGGGAATCAATTTCACTGATGGATCTGTTGAGGTAT
  I  H  R  T  Y  L  G  G  G  I  N  F  T  D  G  S  V  E  V  L

         730                 750                 770
           .         .         .         .         .         .
TGGCCGCGACACAAATGCCTGGGGAGGCTGCTGGATGGTTCCGCGGAACAGCGGATGCCG
  A  A  T  Q  M  P  G  E  A  A  G  W  F  R  G  T  A  D  A  V

         790                 810                 830
           .         .         .         .         .         .
TCAGAAAATTTATCTGGGTGCTTGAGGACTACTATAAGCATAAATCCATAGAGCACATTT
  R  K  F  I  W  V  L  E  D  Y  Y  K  H  K  S  I  E  H  I  L

         850                 870                 890
           .         .         .         .         .         .
TGATCTTGTCGGGCGATCAGCTTTATCGCATGGATTACATGGAGCTTGTGCAGAAACATG
  I  L  S  G  D  Q  L  Y  R  M  D  Y  M  E  L  V  Q  K  H  V

         910                 930                 950
           .         .         .         .         .         .
TGGATGACAATGCTGACATTACTTTATCATGTGCCCCTGTTGGAGAGAGCCGGGCATCTG
  D  D  N  A  D  I  T  L  S  C  A  P  V  G  E  S  R  A  S  E
         970                 990                1010
           .         .         .         .         .         .
AGTACGGGCTAGTGAAGTTCGACAGTTCAGGCCGTGTGATCCAGTTTTCTGAGAAGCCAA
  Y  G  L  V  K  F  D  S  S  G  R  V  I  Q  F  S  E  K  P  K

        1030                1050                1070
           .         .         .         .         .         .
AGGGCGACGATCTGGAAGCGATGAAAGTGGATACCAGTTTTCTCAATTTCGCCATAGACG
  G  D  D  L  E  A  M  K  V  D  T  S  F  L  N  F  A  I  D  D

        1090                1110                1130
           .         .         .         .         .         .
ACCCTGCTAAATATCCATACATTGCTTCGATGGGAGTTTATGTCTTCAAGAGAGATGTTC
  P  A  K  Y  P  Y  I  A  S  M  G  V  Y  V  F  K  R  D  V  L
```

```
1150                1170                1190
TGCTGAACCTTCTAAAGTCAAGATACGCAGAACTACATGACTTTGGGTCTGAAATCCTCC
 L  N  L  L  K  S  R  Y  A  E  L  H  D  F  G  S  E  I  L  P

      1210                1230                1250
CGAGAGCTCTGCATGATCACAATGTACAGGCATATGTCTTCACTGACTACTGGGAGGACA
 R  A  L  H  D  H  N  V  Q  A  Y  V  F  T  D  Y  W  E  D  I

      1270                1290                1310
TTGGAACAATCAGATCCTTCTTCGATGCGAACATGGCCCTCTGCGAACAGCCTCCAAAGT
 G  T  I  R  S  F  F  D  A  N  M  A  L  C  E  Q  P  P  K  F

      1330                1350                1370
TTGAATTTTATGATCCAAAAACCCCCTTCTTCACTTCGCCTCGGTACTTACCGCCAACAA
 E  F  Y  D  P  K  T  P  F  F  T  S  P  R  Y  L  P  P  T  K

      1390                1410                1430
AGTCAGACAAGTGCAGGATCAAAGAAGCGATCATTTCGCACGGCTGCTTCTTGCGTGAAT
 S  D  K  C  R  I  K  E  A  I  I  S  H  G  C  F  L  R  E  C

      1450                1470                1490
GCAAAATCGAGCACTCCATCATCGGCGTTCGTTCACGCCTAAACTCCGGAAGCGAGCTCA
 K  I  E  H  S  I  I  G  V  R  S  R  L  N  S  G  S  E  L  K

      1510                1530                1550
AGAACGCGATGATGATGGGCGCGGACTCGTACGAGACCGAGGACGAGATCTCGAGGCTGA
 N  A  M  M  M  G  A  D  S  Y  E  T  E  D  E  I  S  R  L  M

      1570                1590                1610
TGTCTGAGGGCAAGGTTCCCATCGGCGTCGGGGAGAACACAAAGATCAGCAACTGCATCA
 S  E  G  K  V  P  I  G  V  G  E  N  T  K  I  S  N  C  I  I

      1630                1650                1670
TCGACATGAACGCGAGGATAGGAAGGGACGTGGTCATCTCAAACAAGGAGGGGGTGCAAG
 D  M  N  A  R  I  G  R  D  V  V  I  S  N  K  E  G  V  Q  E

      1690                1710                1730
AAGCCGACAGGCCGGAGGAAGGGTACTACATCAGGTCCGGGATCGTGGTGATCCAGAAGA
 A  D  R  P  E  E  G  Y  Y  I  R  S  G  I  V  V  I  Q  K  N
```

```
1750                1770                1790
ACGCGACCATCAAGGACGGCACCGTCGTGTAGGGCGTGCCCGGGTCGGCGCGACGGGGTTC
  A  T  I  K  D  G  T  V  V  *

      1810                1830                1850
TGCGACAACCTGTGCCGCTGCGTCGGTCGTCATCATCTTCTCAAACTCCGGGACTGAAGAA

      1870                1890                1910
GTGATCCGGGGACGGGAGACGTTTGAAGCTTGAATGACTGAGACTGAAAGTGAAGGCGCA

      1930                1950                1970
GCAGAGGCAGGCAGCATTAGTAGTAAGTAGTAAGTAAGTAGCAGTGGAACAAAGTAATAG

      1990                2010                2030
TCGTTCGTTTTTCCCCTGTAATAAATAAGAGGCTGTG.GTTGAGGTAAAAAAAAAAA
```

FIG. 15

Nucleotide sequence and derived amino acid sequence of a cDNA encoding the small subunit of ADP-glucose pyrophosphorylase from barley seed endosperm.

```
           10                  30                  50
            .         .         .         .         .         .
AAAAGTGAACTCACACATCACTCAATATCTATATCCTTCCATTTTATATCCCTCGGTGAT

           70                  90                 110
            .         .         .         .         .         .
GGATGTACCTTTGGCATCTAAAGTTCCCTTGCCCTCCCCTTCCAAGCATGAACAATGCAA
 D  V  P  L  A  S  K  V  P  L  P  S  P  S  K  H  E  Q  C  N

          130                 150                 170
            .         .         .         .         .         .
CGTTTATAGTCATAAGAGCTCATCGAAGCATGCAGATCTCAATCCCCATGCTATTGATAG
 V  Y  S  H  K  S  S  S  K  H  A  D  L  N  P  H  A  I  D  S

          190                 210                 230
            .         .         .         .         .         .
TGTTCTCGGTATCATTCTTGGAGGTGGTGCAGGGACTAGATTGTATCCCCTGACGAAGAA
 V  L  G  I  I  L  G  G  G  A  G  T  R  L  Y  P  L  T  K  K

          250                 270                 290
            .         .         .         .         .         .
GCGTGCAAAGCCTGCAGTGCCATTGGGTGCCAACTACAGGCTTATTGATATTCCTGTCAG
 R  A  K  P  A  V  P  L  G  A  N  Y  R  L  I  D  I  P  V  S

          310                 330                 350
            .         .         .         .         .         .
TAATTGTCTGAACAGCAACATATCAAAGATCTATGTGCTTACACAGTTCAACTCAGCTTC
 N  C  L  N  S  N  I  S  K  I  Y  V  L  T  Q  F  N  S  A  S

          370                 390                 410
            .         .         .         .         .         .
TCTTAATCGTCATCTCTCACGAGCCTATGGGAGCAACATTGGAGGTTACAAGAATGAAGG
 L  N  R  H  L  S  R  A  Y  G  S  N  I  G  G  Y  K  N  E  G

          430                 450                 470
            .         .         .         .         .         .
ATTTGTTGAAGTCCTTGCTGCACAGCAGAGCCCAGATAACCCTGACTGGTTCCAGGGTAC
 F  V  E  V  L  A  A  Q  Q  S  P  D  N  P  D  W  F  Q  G  T

          490                 510                 530
            .         .         .         .         .         .
TGCAGATGCTGTAAGGCAGTACTTGTGGCTATTCGAGGAGCATAATGTTATGGAGTATCT
 A  D  A  V  R  Q  Y  L  W  L  F  E  E  H  N  V  M  E  Y  L
```

```
      550               570               590
        .         .         .         .         .         .
AATTCTTGCTGGAGATCACCTGTACCGAATGGACTATGAAAAGTTTATTCAGGCACACAG
 I  L  A  G  D  H  L  Y  R  M  D  Y  E  K  F  I  Q  A  H  R

      610               630               650
        .         .         .         .         .         .
AGAAACGGATGCTGATATTACTGTTGCTGCCTTGCCCATGGATGAGGAACGTGCAACTGC
 E  T  D  A  D  I  T  V  A  A  L  P  M  D  E  E  R  A  T  A

      670               690               710
        .         .         .         .         .         .
ATTTGGCCTTATGAAAATCGATGAAGAAGGGAGGATAATTGAATTCGCAGAGAAACCAAA
 F  G  L  M  K  I  D  E  E  G  R  I  I  E  F  A  E  K  P  K

      730               750               770
        .         .         .         .         .         .
AGGAGAACAGTTGAAAGCTATGATGGTTGATACGACCATACTTGGCCTTGAAGATGCGAG
 G  E  Q  L  K  A  M  M  V  D  T  T  I  L  G  L  E  D  A  R

      790               810               830
        .         .         .         .         .         .
GGCAAAGGAAATGCCTTATATTGCTAGCATGGGTATCTATGTTATTAGCAAACATGTGAT
 A  K  E  M  P  Y  I  A  S  M  G  I  Y  V  I  S  K  H  V  M

      850               870               890
        .         .         .         .         .         .
GCTTCAGCTTCTCCGTGAGCAATTTCCTGGAGCTAATGACTTCGGAAGTGAAGTTAT.CC
 L  Q  L  L  R  E  Q  F  P  G  A  N  D  F  G  S  E  V  X  P

      910               930               950
        .         .         .         .         .         .
TGGTGCAACTAGCACTGGCATGAGGGTACAAGCATACCTATACGACGGTTACTGGGAAGA
 G  A  T  S  T  G  M  R  V  Q  A  Y  L  Y  D  G  Y  W  E  D

      970               990              1010
        .                   .                   .
TATTGGTACAATTGAGGCATTCTATAATGCAAATTTGGGAATTACCAAAAAACCAATACC
 I  G  T  I  E  A  F  Y  N  A  N  L  G  I  T  K  K  P  I  P

     1030              1050              1070
        .         .         .         .         .         .
TGATTTCAGTTTCTATGACCGTTCTGCTCCCATTTACACACAACCTCGACACTTGCCTCC
 D  F  S  F  Y  D  R  S  A  P  I  Y  T  Q  P  R  H  L  P  P

     1090              1110              1130
        .         .         .         .         .         .
TTCAAAGGTTCTTGATGCTGATGTGACAGACAGTGTAATTGGTGAAGGATGTGTTATTAA
 S  K  V  L  D  A  D  V  T  D  S  V  I  G  E  G  C  V  I  K
```

```
       1150              1170              1190
          .        .         .        .         .        .
AAACTGCAAGATACACCATTCAGTAGTTGGACTCCGTTCCTGCATATCTGAAGGTGCAAT
 N  C  K  I  H  H  S  V  V  G  L  R  S  C  I  S  E  G  A  I

       1210              1230              1250
          .        .         .        .         .        .
AATAGAGGACACGTTGCTAATGGGTGCGGACTACTATGAGACTGAAGCTGATAAGAAACT
I  E  D  T  L  L  M  G  A  D  Y  Y  E  T  E  A  D  K  K  L

       1270              1290              1310
          .        .         .        .         .        .
CCTTGCTGAAAAAGGTGGCATTCCCATTGGTATTGGAAAGAATTCACACATCAAAAGAGC
 L  A  E  K  G  G  I  P  I  G  I  G  K  N  S  H  I  K  R  A

       1330              1350              1370
          .        .         .        .         .        .
AATCATTGACAAGAATGCTCGTATTGGAGATAACGTGATGATAATCAATGTTGACAATGT
 I  I  D  K  N  A  R  I  G  D  N  V  M  I  I  N  V  D  N  V

       1390              1410              1430
          .        .         .        .         .        .
TCAAGAAGCGGCGAGGGAGACAGATGGATACTTCATCAAAAGTGGCATCGTAACTGTGAT
 Q  E  A  A  R  E  T  D  G  Y  F  I  K  S  G  I  V  T  V  I

       1450              1470              1490
          .        .         .        .         .        .
CAAGGATGCTTTACTCCCTAGTGGAACAGTCATATGAAGCAGATGTGAAATGTATGCCAA
 K  D  A  L  L  P  S  G  T  V  I  *

       1510              1530              1550
          .        .         .        .         .        .
AAGACAGGGCTACTTGCGTCAGTCTGGAATCAACCAACAAGGCCGCGAAGGAGATCATAA

      1570              1590              1610
          .        .         .        .         .        .
AATAAAAA.GGAGTGCCATGCGAGTCACTTCTACACCCTTTTCCCCCCTTGATGTATTAG

       1630              1650              1670
          .        .         .        .         .        .
GAACTGTGATGTACAAGCAACTGTGATGCACTTACGCGAAGTGCCCCTGGATTCAGCTTT

       1690              1710              1730
          .        .         .        .         .        .
CTCTTTGCTTGTAACTGGTTTCCAGCAGACCATGCTATTTGTTGTATGGTTCGTGCAAAA
        1750              1770              1790
          .        .         .        .         .        .
CCTTGCGATGCTTTATATATGCTTTATATATAAACAAGATGAATCCCCGCGCGTTGCTGC
        1810
          .        .
GGCACAAAAAAAAAAAAAAAAAA
```

FIG. 16

Nucleotide sequence and derived amino acid sequence of a cDNA encoding a potato starch branching enzyme.

```
                                                          60
CCCGTCTGTAAGCATCATTAGTGATGTTGTTCCAGCTGAATGGGATGATTCAGATGCAAA

                                                         120
CGTCTGGGGTGAGAACATACAAGAAGGCAGCAGCTGAAGCAAAGTACCATAATTTAATCA

                                                         180
ATGGAAATTAATTTCAAAGTTTTATCAAAACCCATTCGAGGATCTTTTCCATCTTTCTCA
M  E  I  N  F  K  V  L  S  K  P  I  R  G  S  F  P  S  F  S

                                                         240
CCTAAAGTTTCTTCAGGGGCTTCTAGAAATAAGATATGTTTTCCTTCTCAACATAGTACT
P  K  V  S  S  G  A  S  R  N  K  I  C  F  P  S  Q  H  S  T

                                                         300
GGACTGAAGTTTGGATCTCAGGAACGGTCTTGGGATATTTCTTCCACCCCAAAATCAAGA
G  L  K  F  G  S  Q  E  R  S  W  D  I  S  S  T  P  K  S  R

                                                         360
GTTAGAAAAGATGAAAGGATGAAGCACAGTTCAGCTATTTCCGCTGTTTTGACCGATGAC
V  R  K  D  E  R  M  K  H  S  S  A  I  S  A  V  L  T  D  D

                                                         420
AATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGACTGAAAATATTGGCCTCCTAAAT
N  S  T  M  A  P  L  E  E  D  V  K  T  E  N  I  G  L  L  N

                                                         480
TTGGATCCAACTTTGGAACCTTATCTAGATCACTTCAGACACAGAATGAAGAGATATGTG
L  D  P  T  L  E  P  Y  L  D  H  F  R  H  R  M  K  R  Y  V
```

FIG. 17

Nucleotide sequence of a tuber specific class I patatin promoter.

60
TTGTTAGTTAATGCGTATTAGTTTTAGCGACGAAGCACTAAATCGTCTTTGTATACTTTC

120
AGTGACACATGTTTAGTGACGACTGATTGACGAAATTTTTTTCGTCTCACAAAATTTTTA

180
GTGACGAAACATGATTTATAGATGACGAAATTATTTGTCCCTCATAATCTAATTTGTTGT

240
AGTGATCATTACTCCTTTGTTTGTTTTATTTGTCATGTTAGTTCATTAAAAAAAAAATCT

300
CTCTTCTTATCAATTCTGACGTGTTTAATATCATAAGATTAAAAAATATTTTAATATATC

360
TTTAATTTAAAGCCACAAAATTTAAATTTCTTCGTTAACATAATTTGTCAAATCAGGCTC

420
AAAGATCGTTTTTCATATCGGAATCAGGATTTTATTTATTCTTTTAAAAATAAAGAGGTG

480
GTGAGCTAAACAATTTCAAATCTCATCACACATATGGGGTCAGCCACAAAAATAAAGAAC

540
GGTTGGAACGGATCTATTATATAATACTAATAAAGAATAGAAAAAGGAAAGTGAGTGAGG

600
TGCGAGGGAGAGAATCTGTTTAATATGCAGAGTCGATCATGTGTCAGTTTTATCGATATG

660
ACTCTGATTTCAACTGAGTTTAAGCAATTCTGATAAGGCGAGGAAAATCACAGTGCTGAA

720
ATCTAGAAAAATCTCATACAGTGAGATAAATCTCAACAAAAACGTTGAGTCCATAGAGGG

780
GGTGTATGTGACACCCAACCTCAGCAAAAGAAAACCTCCCCTCAAGAAGGACATTTGCGG

840
TGCTAAACAATTTCAAGTCTCATCACACATATATATTATATAATACTAATAAAGAATAGA

900
AAAAGGAAAGGTAAACATCACTAATGACAGTTGCGGTGCAAAGTGAGTGAGATAATAAAC

960
ATCAGTAATAGACATCACTAACTTTTATTGGTTATGTCTTTCTCAAAATAAAATTTCTCA

1020
ACTTGTTTACGTGCCTATATATACCATGCTTGTTATATGCTCAAAGCACCAACAAAATTT

1047
AAAAACACTTTGAACATTTGCCCCGGG

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01082

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/82 C12N15/52 A01H5/00 A23L1/216 A23L1/217
A23L1/29

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A01H A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PLANT PHYSIOL., vol.101, January 1993 pages 179 - 186 KLECZKOWSKI, L.A., ET AL. 'Insensitivity of barley endosperm ADP-glucose pyrophosphorylase to 3-phosphoglycerate and orthophosphate regulation' see the whole document ---	30
X	EMBL SEQUENCE DATABASE ACC. NO. X66080 REL. 31; 13-MAY-1992 T. aestivum AGP-S mRNA ---	26
X	SWISSPROT PROTEIN SEQUENCE DATABASE ACC. NO. P30523; REL. 25; 1-APRIL-1993 GLUCOSE-1-PHOSPHATE ADENYLYLTRANSFERASE SMALL SUBUNIT PRECURSOR ---	27

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
3 April 1995	04.05.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Maddox, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PLANT PHYSIOL., vol.100, 1992 pages 1617 - 1618 VILLAND, P., ET AL. 'ADP-glucose pyrophosphorylase large subunit cDNA from barley endosperm' cited in the application see the whole document	1-13, 17-27, 39,40, 44-46
Y	PLANT MOLECULAR BIOLOGY, vol.19, 1992 pages 381 - 389 VILLAND, P., ET AL. 'PCR amplification and sequences of cDNA clones for the small and large subunits of ADP-glucose pyrophosphorylase from barley tissues' cited in the application see the whole document	1-13, 17-27, 39,40, 44-46
Y	WO,A,91 19806 (MONSANTO) 26 December 1991 cited in the application	1-13, 17-27, 39,40, 44-46
	see page 10, line 28 - page 11, line 14 see page 16, line 11 - page 28, line 27	
Y	MOLECULAR AND GENERAL GENETICS, vol.225, 1991, BERLIN DE KLÖSGEN, R.B., ET AL. 'Subcellular location and expression level of a chimeric protein consisting of the maize waxy transit peptide and the beta-glucuronidase of Escherichia coli in transgenic potato plants'	41
A	see the whole document	13
Y	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol.181, no.1, 1991, DULUTH, MINNESOTA US pages 87 - 94 BABA, T., ET AL. 'Sequence conservation of the catalytic regions of amylolytic enzymes in maize branching enzyme-I' see page 93, last paragraph	41
O,P, X	PLANT PHYSIOLOGY SUPPLEMENT, vol.102, no.1, May 1993 page 47 KLECZKOSKI, L.A., ET AL. 'Isozymes of barley ADP-glucose pyrophosphorylase' see abstract 249	27

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01082

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOL. GEN. GENET., vol.230, 1991 pages 39 - 44 KOSSMANN, J., ET AL. 'Cloning and expression analysis of a potato cDNA that encodes branching enzyme: evidence for co-expression of starch biosynthetic genes' see the whole document	28,29
X	WO,A,92 14827 (IFGFB) 3 September 1992 see the whole document	28,29
P,X	PLANT PHSIOLOGY, vol.102, July 1993 pages 1053 - 1054 POULSEN, P., ET AL. 'Starch branching enzyme cDNA from Solanum tuberosum' see the whole document	28,29
P,X	EMBL SEQUENCE DATABASE ACC. NO. X69805. rel. 36. 31-7-93 S. tuberosum mRNA for starch branching enzyme	28,29
A	PLANT PHYSIOLOGY, vol.101, January 1993 pages 237 - 243 KRAM, A.M., ET AL. 'Localization of branching enzyme in potato tuber cells with the use of immunoelectron microscopy' see the whole document	41-43
A	SCIENCE, vol.258, 9 October 1992 pages 287 - 292 STARK, D.M., ET AL. 'Regulation of the amount of starch in plant tissues by ADP glucose pyrophosphorylase' see the whole document	1-27,30, 39,40, 44-46
A	WO,A,92 11382 (CALGENE) 9 July 1992 cited in the application see page 14, line 1 - line 11	1-27,30, 39,40, 44-46
A	EP,A,0 368 506 (ICI) 16 May 1990 cited in the application see column 4, line 39 - line 43	1-27,30, 39

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01082

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PLANT MOLECULAR BIOLOGY, vol.12, 1989 pages 525 - 538 OLIVE, M., ET AL. 'Isolation and nucleotide sequences of cDNA clones encoding ADP-glucose pyrophosphorylase polypeptides from wheat leaf and endosperm' cited in the application see the whole document	1-27
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol.264, 1989, BALTIMORE, MD US pages 12238 - 12242 ANDERSON, J.M., ET AL. 'The encoded primary sequence of a rice seed ADP-glucose pyrophosphorylase subunit and its homology to the bacterial enzyme' see the whole document	27
E	DATABASE WPI Section Ch, Week 9419, Derwent Publications Ltd., London, GB; Class C06, AN 94-155835 & JP,A,6 098 656 (MITSUI GYOSAI SHOKUBUTSU BIO KENKYUSHO) 12 April 1994 see abstract	41
L	FEBS LETTERS, vol.332, October 1993 pages 132 - 138 KHOSHNOODI, J., ET AL. 'Characterization of the 97 and 103kDa forms of starch branching enzyme from potato tubers' see results section 3.2 and reference 22 and 23 .This document is cited to establish the structure of the clone BE7 reported in MGG 230:39-44	28,29

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INTERNATIONAL SEARCH REPORT	International application No. PCT/EP 94/01082

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.- claims 1-27,30-36,39,40,44-46: Increasing rate/yield of starch production in transgenic plants using barley endosperm AGP.
2.-claims 28,29,41-43: Intracellular location of transgene products to the amyloplast using starch branching enzyme tansit peptide.
3.- claims 37,38: Provision of plant transformation vector.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-36,39-46

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 94/01082

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